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SOME EFFECTS OF SOLID ROCKET MOTOR FUEL EXHAUSTS ON AVIAN EMBRYOS

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ABSTRACT

Fertile White Leghorn (Gallus gallus) and Bobwhite Quail (Colinus virginianus) were subjected to 15 min exposures produced by burning solid rocket motor (SRM) fuel. Comparative mortality data were collected. Chicken eggs were further used to study the effects of exposure on water relations and blood gas parameters.

Chicken embryos exposed once on day 19 of incubation demonstrated an LD_{50} of 204 ppm; the LD_{50} for quail embryos was 175 ppm. When mortality was regressed on the final exposure concentration, chicken and quail embryos exposed on days 12 and 19 showed LD_{50}'s of 127 and 86 ppm respectively, and embryos exposed on days 4, 12, and 19 had LD_{50}'s of 75 and 56 ppm. Quail embryos appeared to be more sensitive to SRM exhausts than chicken embryos, probably owing to the larger surface area to volume ratio of the egg. Embryos exposed to a small daily concentration had an MLC of 117 ppm and an LD_{50} of approximately 200 ppm for cumulative exposure concentrations. This suggested that individual exposures were additive in effect. Eggs exposed at temperatures less than 37.5°C showed reduced lethality, while exposures at greater temperatures increased lethality.

The rate of water loss from chicken eggs measured over an eight hour period increased 5 times because of a 15 min exposure. Since the increased rate of dehydration occurred during the exposure, the hydroscopic effects of exposure were extreme. When

eggs lose the 18% of the initial weight normally lost from evaporation during incubation due to exposure, no more water loss was seen to occur.

Blood gas analyses on 12 day embryos showed decreased pH at cumulative exposure concentrations greater than 200 ppm. Carbon monoxide in the exhausts probably increased carboxyhemoglobin, reducing buffering capacity. This acidosis was partially compensated by increased HCO_3^-. Exogenously derived Cl^-, plus increased HCO_3^- may shift intracellular K^+, making the serum hyperkatremic. Dehydration effects further increased serum hypertonicity.

INTRODUCTION

The use of solid rocket motor (SRM) fuels has raised the question of possible environmental perturbation caused by the release of the exhaust products (Nimmo et al. 1974; Stout et al. 1974). During rocket launches, enormous quantities of at least eight types of exhaust products (Table 1) are dispersed at or near ground level (Stout et al. 1974; NASA anon undated). Some aspects of the ecological effects on various biotic and abiotic systems and the environmental fate of these exhausts have been studied (Nimmo et al. 1974; Stout et al. 1974). However, since these studies have raised many questions and left others unanswered, additional study of the biological effects of SRM exhaust is warranted.

The Kennedy Space Center will function as the primary site for space shuttle launchings and, therefore, will be the area most exposed to exhausts from rockets using solid fuel boosters. Merritt Island National Wildlife Refuge surrounds the Kennedy Space Center. This preserve is a well known nesting and wintering area for many avian species. Since birds comprise both esthetic and ecological components of the refuge, they were selected for critical study.

In the avian life cycle, incubation and embryonic development are most sensitive to environmental influence. Furthermore, bird embryos are amenable to laboratory study and an extensive literature exists from their widespread use in both the pure and applied sciences. For these reasons the avian embryo has been selected for

the study of SRM exhaust toxicity.

This study examined the mortality of domestic chicken (Gallus gallus) and bobwhite quail (Colinus virginianus) embryos exposed to various concentrations and numbers of exposures of SRM exhausts. The effect of exposure to SRM exhausts on several blood parameters was measured and evaluated; a possible mechanism causing death is postulated.

LITERATURE REVIEW

Solid Rocket Motor Fuel Exhausts

To assess the effects of SRM exhausts on bird embryos, it is necessary to examine the exhaust composition and to consider the potential effects of each component on the organism. The exhaust composition and mechanism of combustion have been reviewed by Vickers (1974). According to Vickers, "the solid fuel rockets of interest are basically powered by the oxidation of metallic aluminum and perchlorate. The fuel consists of about 16% by weight aluminum and 68% ammonium perchlorate embedded in an organic matrix." The theoretical combustion products, which account for 99% of the original fuel, are in Table 1. In addition to the theoretical products listed, monitoring of an actual launch demonstrated that small measureable quantities of $AlCl_3$ and $FeCl_2$ were present in the exhaust.

Although hydrogen gas is the most abundant theoretical exhaust product, the biological effect of the gas on avian embryos is probably not significant in nature. This effect is due to the tendency of the gas to rapidly rise and disperse with the surrounding air.

Gaseous water and nitrogen released as a result of combustion are necessary or innocuous to avian embryos. H (gas) and Cl^- (gas) probably exert minimal effects due to their low concentrations (Table 1) and to their short chemical half-lifes (Vickers 1974).

Table 1. Major theoretical combustion products of solid rocket motor fuel and the weight of these products during the launch of a Titan III-C booster.

Product	Form	Mole Fraction	Kg per Launch	
		Vickers 1974	NASA Anon Undated	Cesta and McLouth 1969
H_2	gas	0.32008	7,582	7,774
CO	gas	0.25761	84,898	98,899
HCl	gas	0.14744	63,106	75,871
H_2O	gas	0.09663	20,657	38,772
N_2	gas	0.07801	2,724	3,795
Al_2O_3	solid	0.07708	95,616	116,878
CO_2	gas	0.01407	8,535	11,332
H	gas	0.00506	-	-
Cl^-	gas	0.00170	-	-
$FeCl_2$	solid	-	1,226	-

Carbon monoxide produces a deleterious effect on developing chicken embryos (Baker and Tumasonis 1972; McGrath and Moffa 1972). Baker and Tumasonis (1972) found that after a 24 hr exposure to 425 ppm of CO, chick embryos showed the greatest mortality between 8 and 17 days of incubation (Table 2). They indicated that for this exposure, 425 ppm seems to be a critical concentration for viability and non-fatal metabolic responses. However, McGrath and Moffa (1972) found that constant exposure to CO levels over 100 ppm decreased hatchability (Table 2).

In SRM exhaust, HCl gas probably dissolves in water, especially at lower ambient temperatures and high humidity, and produces a strong acid (Rhein 1973). Several investigators have studied the effect of HCl gas on mammalian systems (Machle et al. 1942; Higgins et al. 1972); however, no studies have been found which discuss the effect of HCl gas on avian systems.

Aluminum oxide (alumina) particles emitted in SRM exhausts are chemically inert and, alone, probably exert little, if any, effects on avian embryos. Alumina particles emitted by the Titan III-C (single motor) booster rocket are spherical solids that average 0.5 μm in diameter (Barker 1965). The particles have large surface areas and are potentially capable of adsorbing H_2O, HCl, CO, and other substances. However, Baldwin (1974) discovered no significant degree of adsorbtivity of the above substances on the alumina particles emitted in SRM exhausts.

Carbon dioxide in SRM exhausts would not be anticipated to have a significant biological effect, since it is a common product

Table 2. Percent hatchability of chicken embryos to various exposure levels of carbon monoxide in two different studies.

Carbon monoxide concentration in ppm	Percent Hatchability	
	Baker and Tumasonis 1972 24 hr exposure	McGrath and Moffa 1972 constant exposure
0	88	79 ± 14
100	--	63 ± 17
200	--	13 ± 13[a]
425	78	--
650	45	--
800	0	--
1000	0	--

[a]Represents percent viability at 15 days of incubation.

from biotic and abiotic oxidations. As an animal waste, CO_2 is harmless unless present in high concentrations. In terrestrial amniotes high CO_2 concentrations cause respiratory acidosis which reduces arterial pH. Generally, exposure to high CO_2 concentrations are not lethal provided sufficient O_2 is present. The cleidoic egg, however, is quite susceptible to elevated CO_2 concentrations, since the concentration within the egg's air cell and serum pCO_2 and HCO_3^- normally increases throughout incubation (Romanoff 1960).

The iron found in the exhaust cloud was probably from eroded rocket pods caused by the high temperature (3255.7°K) and pressure (26.8 atm) produced by the combustion of rocket ignition (Vickers 1974). The ferrous chloride may go into solution or disperse as particulate matter in the exhaust cloud. Atmospheric iron from SRM exhausts is important because it could interfere with the embryos resistance to microbial infection. In the embryo, albumen is a major defense against microbial infection. Antimicrobial components of albumen include lysozyme, conalbumen, and riboflavin (Board 1966). Garibaldi (1960 from Board 1966) has shown that microbial growth inhibition by the egg white is extensively overcome by adding iron. Board (1964) demonstrated bacterial multiplication following the addition of iron to either shell membranes or the albumen of intact eggs.

Incubation Parameters

Growth and development of the avian embryo are influenced by intrinsic genetic endowment, maternal effects, and extraembryonic influences. Temperature, humidity, barometric pressure, physical

turning and handling, noise, and atmospheric composition are factors which can effect the developing embryo. Romanoff (1972) and Landauer (1960) have reviewed the effects of many of these factors on avian development.

Temperature

Temperature is the most important factor influencing embryonic development, since it controls onset of development (Romanoff 1972; Landauer 1960; Barott 1937). The physiological zero (i.e. the minimum temperature required to initiate development) has been variously placed from 20.7°to 30°C (Romanoff 1972). The optimum incubation temperature is dependent upon humidity (Landauer 1960), but is generally accepted to be from 37.5°to 37.8°C for chickens and quail (Romanoff 1972; Roseberry and Klimstra 1965). Barott (1937) determined that at 60% relative humidity and O_2 concentration 21%, the optimum hatching temperature for White Leghorn chickens was 37.9°C with the air movement past the eggs equal to 0.57 m^3 per min. Sensitivity to cold varies with the size of the egg (Landauer 1960), length of exposure (Romanoff 1960; 1972), species (Batt and Cornwell 1972; Romanoff 1943a), and age of the embryo (Aulie and Moen 1975; Bell and Freeman 1971). Within the brief range of above optimum temperatures that permit development, growth is accelerated (Barott 1937), although the stimulating effect of elevated temperatures is less (per degree) than the retarding effect of suboptimal temperatures (Romanoff 1960).

Humidity

Humidity is tantamount to temperature for normal development

and influences the optimal temperature (Landauer 1960). Developing embryos lose weight at a constant rate during incubation; most of this loss is water vapor (Rahn and Ar 1974; Boone and Barmore 1965). The rate of weight loss is directly dependent upon relative humidity at constant temperature and this influences hatchability.

Low humidity hastens growth and reduces chick weight while high humidity has the reversed effects (Romanoff 1972; Barott 1937). Romanoff (1972) further states that "surviving embryos incubated under high humidity become highly susceptible (to death), and many die within one week after hatching." Landauer (1960) has reviewed the effects of various humidities. He cited studies by Callenback and Hiller (1934) and Romanoff (1934) that indicated pheasant eggs require higher humidity toward the end of incubation, although chickens and quail seem to require lower humidity during the same period. Small eggs require a higher optimum humidity (Marshall and Cruickshank 1938); presumeably the higher surface area to weight ratio causes these eggs to lose weight more rapidly than large ones. Water vapor permeability (cm^3 per STP per cm per torr per sec), conductance (mg per day per torr) through the shell (Ar et al. 1974), incubation time (Rahn and Ar 1974), and environmental adaptations (Wangensteen et al.1974) are other factors which affect water loss. Barott (1937) tested for the effect of humidity on hatchability. He reported that optimum hatches were obtained at 55 to 65% relative humidity. He also found that slightly higher incubation temperatures gave optimum hatch at slightly lower relative humidities.

Orientation and Turning

The orientation and turning (or lack thereof) of pre-incubated eggs has no influence on hatchability (Proudfoot 1969). However, position and rotation of incubating eggs influences hatchability. Landauer (1960) concludes that: 1) hatchability is highest when eggs are incubated large end up; 2) turning of eggs is critical to successful hatch, with successively improved hatchability as a result of increasing the number of turns from two to eight in 24 hrs; more than eight turns yielded no increase in hatchability; 3) when turned mechanically, rotating eggs 40°to 45° gives better hatchability than when rotating through 30°. Robertson (1961a from Romanoff 1972) presented data which show that embryonic mortality at hatching continues to decrease when eggs are turned up to 144 turns per day.

Noise

The noise and vibration of a rocket launch have raised the question of the effect of these parameters on hatchability. Although outside the scope of this study, this question may be answered in part from Landauer (1960): "... Fronda (1938) found that an earthquake of intensity VI-VII had no detrimental effect on hatchability, irrespective of whether the eggs at the time of the earthquake were in the first, second, or third week of incubation. Sound intensities of 96 decibels had no measureable effect on hatchability (Stadelman 1958). When cocks were exposed to intermittent noises (intensity 120 decibels) and their semen was used for artificial insemination, Kosin (1958a) found a significant

lowering of hatchability in one trial, but not in a second one. It is well recognized, of course, that significant differences may arise by chance."

Atmospheric Composition

Atmospheric composition exerts a profound effect on avian hatchability. During incubation the availability of sufficient O_2 and the removal of excessive CO_2 are necessary for successful hatching. In addition to these gases, the effects of others such as N_2, He, natural gas, N_2O, polluted air, and formaldhyde have been tested on avian embryos. The most important of these are discussed below.

Carbon Dioxide - Lamson and Edmond (1914) found no effect of CO_2 concentration up to 1.5%, after which increased concentrations result in markedly decreased hatches with the extinction point (i.e. 100% mortality) being about 2%. Riddle (1924) demonstrated for ringdove and common pigeon embryos that developmental stage was the most important factor in the death or survival of embryos placed in atmospheres of altered O_2 and CO_2 composition. Although a few embryos survived 8 to 12% CO_2 for 24 hrs, all were killed when the concentration was 33 to 46% (O_2 was 51%). Riddle (1924) states that atmospheric CO_2 can be increased to several times its normal value, regardless of whether the O_2 is increased or slightly decreased without injury to embryos.

Romanoff (1930) found that about 0.4% CO_2 stimulated the growth of chicken embryos during the first few days of incubation. Amounts of CO_2 above 1% with reduced O_2 resulted in slow growth,

formation of abnormalities, and early embryonic death. Exposures to 10.0% CO_2 and 18% O_2 at 24 and 48 hr intervals resulted in smaller embryos. Romanoff (1930) found 100% mortality with continuous incubation at 16.3% O_2 and 22% CO_2. Romanoff (1972) further stated that susceptibility to elevated CO_2 is markedly higher at 0 to 4 days of age, with tolerance of higher levels of CO_2 increasing throughout incubation.

Barott (1937) found that an increase of 1% in CO_2 reduced hatchability of chickens about 15%. He showed that a gradual increase of naturally produced CO_2 to a level of 5.5% by 10 days of incubation was not as lethal as constant exposure to 4% CO_2. These observations provide some support to those of Romanoff (1972).

Taylor et al. (1956) observed the effect of exposure to various CO_2 tensions during the first 96 hrs of incubation. They found normal hatchability when CO_2 levels were from 0 to 1%, and that hatchability decreased inversely when CO_2 tension was increased from 1.1% to an extinction level of 7.5%. Contrary to earlier reports (Romanoff 1930; Sadler et al. 1954), but in agreement with Romanoff (1972), hatchability is not improved by increased CO_2 concentrations of 2 to 4% during the first 48 hrs of incubation. Taylor et al. (1956) found that mild O_2 and CO_2 stresses were additive in reducing hatchability, but that severe stresses acted synergistically to produce greater than expected mortality.

Taylor and Kreutziger (1964) continued the above study by exposing 5-to-8-day embryos. Compared to the 0 to 4 day exposures, 5-to-8-day old embryos demonstrated an increased

tolerance to elevated CO_2. Normal hatches occurred after exposure to 0 to 3% CO_2 and 16 to 45% O_2. Extinction levels occurred for CO_2 at 8%, and for O_2 at 12.5 and 82%. As reported earlier by Taylor et al. (1956), synergism of lethal effects occurred at the more severe levels of both gases.

Taylor and Kreutziger (1966) studied hatchability effects of various levels of CO_2 and O_2 exposure from the 9th through the 12th days of incubation. Their data demonstrate some changes in tolerance when compared to exposure at earlier stages of development. The authors discuss physical and physiological changes in the egg-embryo system which occur during the 9th through the 12th days of incubation to alter the response to gas tensions. They show that the combination of these changes, which take place in the extra embryonic membranes and the albumen and yolk cause them to undergo further modification which makes the egg-embryo system more resistant to acidosis. Eggs exposed during the 9th through the 12th day period show continued increase in tolerance to CO_2; no reduction in hatchability occurred up to 6% CO_2 with O_2 levels between 15 and 16%.

Oxygen - During the first five days of development of the chicken embryo hatchability is highest at 32% O_2 (Cruz and Romanoff 1944). Teratogenic effects occur at less than 12% O_2 with sensitivity to hypoxia increasing with age (Grabowski and Paar 1958). Allen (1961) discovered that incubation in 100% O_2 during this period results in failure to develop an evident heart beat. Five-to-eight-day old embryos demonstrate an increased sensitivity to a

high O_2 level (Taylor and Kreutziger 1964), but resistance continued to improve after this time (Taylor and Kreutziger 1966). Barott (1937) reported that hatchability decreased about 1% for each increase of 1% in O_2 concentration between 30 and 50%; each decrease of 1% below 21% decreased hatchability by 5%. Noguchi (1969) studied the teratogenic effects of hypoxia. Using exposure periods of three hours, he found a linear relationship between the age of the exposed embryo and the percent of O_2 which yielded LD_{50}s.

Helium - Several investigators have examined the effects of incubating chick embryos in a 79% He:21% O_2 atmosphere (Weiss and Wright 1968; Ferguson et al. 1973). Although Weiss and Wright (1968) observed that hatchability was inversely related to the length of exposure in He:O_2, the differences in hatchability between experimental and control eggs have been attributed to variation in the incubators used for experimental and control eggs (Ferguson et al. 1973), and to other parameters such as humidity and temperature. Valera (1968) points out that the thermal conductivity of the He:O_2 atmosphere is greater than that of ambient air, thus egg temperature of eggs incubated in He:O_2 are lower than in ambient air. When relative humidity is increased in He:O_2 from the normal 55 to 60% up to 75 to 85% relative humidity, hatchability has been variously reported not to change (Weiss and Wright 1968) or to improve from 3.3 to 71.9% and 19.4 to 63.6% Ferguson et al. 1973). Other changes in exposed and control eggs which were found in the above studies were: 1) an increase in embryonic metabolism (Weiss et al. 1965 from Romanoff 1972); 2)

a decrease in embryonic metabolism (Ferguson et al. 1973); and 3) no gross or histopathologic changes (Yalera 1968; Ferguson et al. 1973).

Nitrous oxide - Rector and Eastwood (1964) incubated chicken eggs in an atmosphere of 80% N_2O and 20% O_2. "Nitrous oxide has long been considered to be the least toxic and most inert of all anaesthetic agents" (Romanoff 1972), although N_2O has been known to inhibit cell growth and cause death in the bone marrow (Rector and Eastwood 1964). Rector and Eastwood (1964) discovered that hatchability was delayed 36 hrs and reduced (controls 50-60%, N_2O 10-20%) in the N_2O treated group. Spastic paralysis was noted in three of the fifteen experimental chicks.

Formaldehyde- As noted by Romanoff (1972) and by Proudfoot and Stewart (1970), numerous investigators have experimented with the effect of formaldehyde and formaldehyde $KMnO_4$ disinfectants on hatchability (Insko et al. 1941). These studies document the concentrations of disinfectant necessary to kill common pathogens, and the sensitivities of chicks to disinfectant at various stages of development (Proudfoot and Stewart 1970). Proudfoot and Stewart (1970) have shown that pre-incubation fumigation with a standard concentration did not affect hatchability, provided the length of fumigation is no longer than 30 min and ventilation is provided following treatment. Concentrations exceeding the above standard, or lengths of exposure over 30 min dramatically increase mortality (Insko et al. 1941).

Shell Permeability

Kutchai and Steen (1971) studied the O_2 and CO_2 permeability of the eggshell and membranes. They found that the permeability of newly laid eggs is not high enough to permit the O_2 uptake rates observed later in development. In fertilized eggs the O_2 permeability increases dramatically between the second and fifth days of incubation while in unfertilized eggs permeability remains low. A similar phenomenon occurs with CO_2. Kutchai and Steen (1971) believed that air filled channels develop during early incubation. Using the permeability data of eggshells without membranes from Wangensteen et al. (1969), they determined that the permeability of the compound membrane to O_2 is about 30 times the permeability of an equivalent layer of water. Thus, they conclude that the membranes must have gas filled channels. Kutchai and Steen (1971) provide evidence for the existence of gas filled pores of newly laid eggs: they calculated the CO_2/O_2 permeability ratio in newly laid eggs to be 3, and in fertilized eggs incubated six days or longer to be 1.3. If the gas transport barrier were aqueous the ratio would be similar to the ratio of the solubilities in water, about 30. Since the CO_2/O_2 permeability ratio is closer to the 0.85 ratio of the gas diffusion coefficients, they conclude that most of the gas flux must occur through gas filled channels. Kutchai and Steen (1971) measured the water content of compound membranes of fertilized eggs and unfertilized eggs and determined there is considerably more drying in membranes from fertilized eggs. They postulate that drying is caused by dehydration from the

increased colloid osmotic pressure of the albumen, and that after the fifth day of development the drier membranes are maintained by physical separation from the egg contents by the underlying allantoic membrane.

Wangensteen et al. (1970/1971) concluded that the principal barrier to O_2 diffusion is the eggshell. They assumed that the inner and outer shell membranes were dry and did not significantly approach the resistance offered by the shell. Consistent with the observations of Romijn and Roos (1938) that the air cell increases in size at a constant rate, Wangensteen et al. (1970/1971) concluded that the permeability of the eggshell to O_2 does not increase with incubation. Romanoff (1943a) found that the driving force across the shell was pressure and not passive diffusion. Wangensteen et al. (1970/1971) noted that the shell is about 100 times more permeable to O_2 than to a layer of stagnant water of the same thickness, and more than 1000 times less permeable than to a comparable layer of stagnant air.

Wangensteen and Rahn (1970/1971) provided more evidence that gas exchange by the avian embryo occurs by means of diffusion through the shell. They show that O_2 diffusion is driven by the metabolic utilization of O_2 by the embryo. Since the shell permeability is constant, the diffuseability of O_2 is determined by the O_2 flux across the shell. As the embryo uses more O_2, the air cell O_2 decreases, which increases the O_2 gradient and the O_2 flux into the egg. Wangensteen and Rahn (1970/1971) point out that this occurs at the expense of arterial O_2 tension and that the

shell permeability thus determines the maximum rate of O_2 consumption.

Romijn's (1950) discussion of gas diffusion through the shell concluded several important points: 1) the amount of gas passing the shell membrane system is directly proportional to the difference in gas pressure on both sides; 2) the thickness of the shell membranes, calculated by subtracting wet and dry membrane diffusabilities, is 70 μm; and 3) that the amount of O_2 diffusing across the portion of the shell covering the air space during the last 24 hrs of incubation is approximately 80% of the total respiratory metabolism. Romijn (1950) found that the permeability of the air space of the infertile egg does not increase as greatly as does that of the fertile egg under the same conditions. He suggests that internal conditions and the atmosphere of the air space itself are of great importance in increasing the permeability of the shell membrane system to gases during incubation. He concluded that the diffusion of CO_2, H_2, O_2, and N_2 is enormously increased by drying.

Romijn and Roos (1938) measured the gaseous composition of the air space during incubation. They noted that O_2 decreases slightly and CO_2 increases slightly during the first 10 days of incubation. Thereafter there is a progressive and rapid temporal change of the concentrations of these gases. Their measurement of O_2 in the air cell showed that it decreased from about 21% at laying to about 12% on day 20. They discovered that the air space pressure remained constant throughout incubation.

Wangensteen et al. (1969) measured the diffusive permeability

of the shell to O_2. Their measurements indicated that average permeability tended to increase slightly with incubation age.

Visschedijk (1968b) measured the effect of artificially changing the permeability of the shell over the air cell at the times of pipping and hatching. He found that decreasing the permeability of the shell to atmospheric gases accelerated the time of pipping, while increasing the permeability delayed pipping. The time of hatching was not affected by either treatment. The stimulation or inhibition of pipping by covering the air cell was not effective until pulmonary respiration had begun. Visschedijk (1968c) stated that the deviations in the length of the parafetal period (i.e. time between onset of pulmonary respiration and pipping of the shell) noted earlier (Visschedijk 1968ab) owing to changes in air space shell permeability were affected about twice as much by elevated CO_2 levels as by decreased O_2 in the air cell.

Romijn (1950) showed that the permeability of the shell of the chick embryo late in development must increase, otherwise only 3% of the amount of O_2 actually consumed would otherwise be permitted to pass. He suggests that the portion of the shell over the air space is responsible for the increase in permeability. As noted above (Visschedijk 1968a; Tazawa 1972), this is not entirely accurate.

Eggshell and Shell Membranes

The structure of the eggshell and shell membranes have been extensively studied (Simkiss and Taylor 1971; Board 1966; Romanoff 1960; Romanoff and Romanoff 1949). Recent scanning electron

microscopy (SEM) studies (Fujii 1974; Fujii and Tamura 1970) have confirmed and extended many of the known structural parameters. Important features of chicken and quail eggshell and shell membrane morphology are summarized in Tables 3 and 4.

Developing embryos derive much of their osteogenic calcium from the shell, an amount estimated for chickens to be about 5% of the shell's weight (Simkiss 1968). Kreitzer (1972) found that the shell of Coturnix thins by 7.3% during incubation as compared to 6.4% in chickens (Vanderstoep and Richards 1969).

Romanoff and Romanoff (1949) point out that shell and membrane thickness is roughly correlated with the size of the egg. Eggshell thickness also seems to be inversely correlated with the combined thickness of the shell membranes (Mahmoud and Coleman 1967). The thickness of the fibers of the shell membranes and the size of the spaces between the fibers is generally correlated with the size of the bird and egg (Becking 1975).

Wolken and Schwertz (1948) determined that the dried membranes contained pores about 1 um diam with a density of 2×10^7 pores per cm^2. They found that dried membranes are extremely permeable to gases and that wet membranes are nearly impermeable. They suggest that during embryonic development, the rate of gas influx is controlled to some extent by moisture content of the shell membranes.

Wangensteen et al. (1970/1971) cite reasons for believing that both the inner and outer shell membranes are relatively dry during incubation: 1) they measured permeability of the eggshell-outer

Table 3. Selected morphological characteristics of the eggshell and membranes of chickens and quail.

Feature	Chicken	Quail
Shell thickness	300-340 μm[a]	152-194 μm[d] bobwhite 210-215 μm[b] Coturnix
Shell weight (dry)	5[a] - 6.4[c] g	0.75 - .84[b] g
Percent shell wt/egg wt	8 - 11[a,c]	7.6 ± .4[b]
Number pores per egg	7000 - 17000[e]	--
Pore size	9 μm (radius)[f]	--
Shell composition	98% $CaCO_3$[g]	--
Pore composition	Protein strands[g]	--
Combined membrane thickness	66-68 μm[d]	62-64 μm[d]

[a] Simkiss and Tyler 1958.
[b] Garrett et al. 1972.
[c] Romanoff and Romanoff 1949.
[d] Mahmoud and Coleman 1967.
[e] Tyler 1953.
[f] Tyler and Geake 1953.
[g] Bell and Freeman 1971.

Table 4. Selected morphological characteristics of the shell membranes and cuticle of chickens.

Feature	Outer Membrane	Inner Membrane	Cuticle
Arrangement	Fibers arranged in six sheets parallel to shell, alternate layers at right angles[a]	Fibers parallel to shell in 2-3 layers[a,b,c]	--
Thickness	50 μm	20-22 μm	variable, about 10 μm[a,b]
Fiber length and diameter	15 x 3 μm[a,b]	23-25 x 1.5 μm[a]	--
Mesh size	Gradient from 10 μm (shell) to 1 μm at inner membrane [a,d]	About 1 μm[d]	--
Composition	Fibers are protein cores covered with a polysaccharide mantle (glycoprotein)[a]	Similar to the outer membrane fibers[a]	90% peptide with galactose, mannose, fucose and hexosamine[a]

[a] Simkiss and Taylor 1971.
[b] Fujii and Tamura 1969.
[c] Romanoff and Romanoff 1949.
[d] Wolken and Schwertz 1948.

membrane to O_2 and found it consistent with gas-filled shell pores and a dry membrane; 2) when the inner membrane is removed, the newly exposed outer shell membrane appeared dry; 3) the permeability of fresh eggshell-outer membranes to O_2 is equivalent to that measured after several days on their apparatus; and 4) the permeability of the eggshell-outer membrane system to O_2 was approximately the same as the permeability of the shell alone to O_2. The latter would not be true if the outer membrane were a water barrier. Since the allantois absorbs fluid from the allantoic sac, it could also serve to keep the adjacent membrane dry.

The eggshell ranges in thickness from 60 μm in hummingbirds to 1950 μm in the ostrich (Romanoff and Romanoff 1949). The hen's eggshell varies from 260 to 370 μm (Romanoff and Romanoff 1949; Carter 1968) and the bobwhite quail eggshell ranges from 152 to 194 μm (Mahmoud and Coleman 1967). Shell thickness is influenced by heredity, the seasons, nutrition, and various physiological factors (Romanoff and Romanoff 1949; Romanoff 1972). Lamova (1971) concluded that an inverse correlation exists between the thickness of the shell and the shell membrane in duck eggs.

The air pore of the shell has been the subject of numerous investigations (Marshall and Cruickshank 1938; Tyler 1953; 1956; 1969; Tyler and Geake 1953; Fujii and Tamura 1970; Becking 1975). Marshall and Cruickshank (1938) suggest that air pores may develop around protein strands that pass from the deep layer of the cuticle to the outer lining membrane of the shell before calcification

occurs. Although various air pore configurations such as oval (Romanoff and Romanoff 1949) or funnel-shaped (Gilbert 1971) have been postulated, Fujii (1974) has recently produced and photographed by SEM casts of air pores which verify similar work produced by Tyler (1953; 1956). The air pores number about 100 to 300 per cm^2. A cast is mushroom-shaped with the stalk being 5 to 23 μm and the head 15 to 65 μm in diam (Becking 1975; Tyler 1956). Although the surface pore structure varies among birds (Becking 1975), Marshall and Cruickshank (1938) showed that the surface plaque, or head of the mushroom cast, is essential to the proper water relations and respiratory gas exchange. The inner organic material is hydroscopic, and by capillary action draws water from the albumen to the increased evaporating surface of the plaque. Protein-filled pores were shown to evaporate water forty times faster from albumen than those from which the organic material has been removed (Marshall and Cruickshank 1938).

The diameter of air pores has been variously estimated. Wangensteen et al. (1970/1971) calculated a mean pore size of 12 to 17 μm. Tyler (1953; 1956) visually estimated pore size to be about 14 μm, while Romanoff and Romanoff (1949) list 10 to 20 μm as the range of pore size.

Microbial Infection

Numerous investigators have demonstrated that a wide variety of microorganisms extragenitally invade the egg (Romanoff and Romanoff 1949). Most of these organisms are air, soil, or water saprophytes (Romanoff and Romanoff 1949; Walden et al. 1956).

Several factors probably combine to enhance the permeability of eggs to microbes. Haines and Moran (1940) suggest that as the freshly laid egg cools, bacteria may be readily drawn through the shell by simple suction, especially if the surface of the egg is moist. Similar results may occur if the eggs are washed in cold water, or if a marked change of the egg's temperature occurs. Microbial motility probably affects the permeability of various organisms. Romanoff and Romanoff (1949) suggest that infection by nonmotile organisms may represent secondary invasion. In eggs which are stored for extended periods, Romanoff and Romanoff (1949) cite research which has shown that the hyphae of molds may facilitate the entrance of bacteria (Zagaevsky and Lutikova 1944). Undoubtedly the most important factor that causes eggs to be susceptible to microbial invasion is water on the egg's exterior (Haines and Moran 1940; Stuart and McNally 1943). Romanoff and Romanoff (1949) state: "Dirty eggs (dirt usually contains moisture), washed eggs, and eggs that have been allowed to 'sweat' all show a higher incidence of rotting... than clean, untreated eggs." Rapid changes in temperature may aid microbial invasion because of condensation produced on the shell surface.

Microbial infection is prevented by the shell and shell membranes. Although the outer membrane is thicker, denser, and weighs more than the inner membranes, the inner membrane is more of a barrier to microbial infection (Walden et al. 1956; Lifshitz et al. 1964). This is probably because of the finer mesh composition of the inner membrane (Lifshitz et al. 1964; Lifshitz and Baker

1964).

The albumen also constitutes an important defense against microbial infection. Bacteriacidal action of albumen depends upon maintaining a high pH (6.4 to 9.0). In this pH range various microbial species can be completely inhibited by making iron unavailable (Romanoff and Romanoff 1949). Adding iron also significantly weakens the antibacterial activity of the albumen (Board 1966).

Water Regulation

Regulation of water loss is nearly tantamount to incubation temperature in its importance to the avian embryo. The humidity of the surrounding air is the principal factor which determines the amount of water evaporated from the egg. Barott (1937) found that during the first week of incubation, water was lost (at 60% RH) at the rate of 30 g per 100 eggs per day. He notes that at the time of hatching, an abrupt release occurs which is 12 to 15 times the earlier rate of water loss.

Romanoff (1967) showed that the average daily weight loss is proportional to the weight of the egg, and that a 60 g egg loses from 0.49 to 0.38 g per day. In development, 18% of the original egg weight is lost (Rahn and Ar 1974). Citing Groebbels (1932), Rahn and Ar (1974) have assumed that average water loss throughout incubation is constant, and is equal to

$$\dot{M}_{H_2O} = 0.015 W^{0.74}$$

where: $\dot{M}_{H_2O}$ = weight loss in mg per day

W = egg weight in g

Kutchai and Steen (1971) postulate that the shell membrane changes apparently play no role in the water economy of the developing egg. They cite evidence that newly laid eggs averaged 59.4 g, 18 day fertilized eggs averaged 48.9 g, while 18 day unfertilized eggs averaged 49.1 g. They state that this weight change mainly reflects water loss, "since at R.Q. = 0.8 the weight loss due to a greater mass of CO_2 being lost than O_2 taken up over the first 18 days of incubation is less than 0.5 g."

Romijn and Roos (1938) studied water loss by measuring the change which occurs in the volume of the air cell during incubation. The air cell increased from several tenths of a cc to an average of 11 cc (corrected to 40°C) after 20 days of incubation.

Wangensteen and Rahn (1970/1971) compare theoretical and actual measurements of water vapor loss. They conclude that if environmental conditions are constant, the fate of the embryo depends entirely upon the permeability of the eggshell. The eggshell must allow sufficient gas exchange for survival yet prevent excessive water loss. Their findings agree with those of Landauer (1960).

Marshall and Cruickshank (1938) have shown that evaporation from the egg is 4.35% of the evaporation from free albumen, and that this difference is caused by the barrier of the shell. They calculate that the pore diameter accounts for 0.115% of this 4.35%, and the surface "plaque" accounts for 1.482% of the demonstrable evaporation. Since these figures account for about one-third of the amount of evaporation which occurs from the egg, some mechanism

must exist for the additional permeability observed in the egg. Marshall and Cruickshank (1938) credit the proteinaceous pore plug and its hydroscopic capillary properties for providing the additional evaporation.

Inconsistent with the conclusions of earlier work (Axelsson 1932) that weight loss and hatchability were negatively correlated, Mueller and Scott (1940) stated that weight loss during the first 24 hrs of incubation was not correlated with hatchability, but was predictive of weight loss during the first 18 days of incubation. However, there was no correlation between egg weight and loss in weight per unit of surface area. Marshall and Cruickshank (1938) demonstrated that small eggs lose weight more rapidly during incubation than large ones and they incorrectly indicated that daily weight loss is nonlinear. Boone and Barmore (1965) confirmed earlier work by showing that daily weight loss is linear, and approximately equivalent for fertile and infertile eggs. Weight loss is primarily a function of egg permeability properties rather than the surface area or volume of the egg (Landauer 1960).

Dunn (1923/1924 from Landauer 1960) stated there are limits to porosity and evaporation beyond which embryonic death occurs, though this is rare and occurs only in visibly abnormal eggs. Hayes and Sumbardo (1926/1927 from Landauer 1960) found that pore density (pores per mm^2) had no effect on hatchability, and Mueller and Scott (1940) state that there is no relationship between porosity and hatchability. Conversely, Axelsson (1932) stated there is significant difference in hatchability between groups that lose

weight at different rates; the lower the weight loss, the higher the percentage of hatchability. This view is adopted by most authors reviewed by Landauer (1960).

Physiological Parameters

Walker (1943) measured the pH changes of allantoic and amniotic fluid on successive days throughout incubation. He found that the allantoic fluid pH is maintained at a fairly constant level through the 13th day of incubation, and he ascribes the pH change during this time to the nature of the renal secretions. Walker (1943) states that "In this connection, Needham (1925; 1926a; 1926b) has shown that there is a succession of end-products of nitrogen metabolism in the chick: first, ammonia is produced, reaching a maximum concentration on the 4th day; secondly, urea, with a maximum on about the 9th day; and finally, uric acid." Walker (1943) points out that dissolved CO_2 in the allantoic (and amniotic fluid probably exerts a considerable effect on its pH and buffering capacity. On day 13, allantoic fluid pH dramatically begins to drop from 7.39 to 5.87 on the 15th day. These pH changes are concommitant with a rapidly increasing evolution of CO_2 beginning about day 10 (Romijn and Roos 1938). Also, a loss of anions in the allantoic fluid occurs because the excretory product being formed is free uric acid rather than its salts. Finally, Walker (1943) points out that calcification of cartilage and osteoid tissue begins about the 15th day of incubation which results in the loss of calcium and phosphorous from extraembryonic fluids with a subsequent reduction in buffering power. Amniotic fluid decreases in pH during the 7 to 15th days of

incubation from about 7.2 to about 6.2. Walker (1943) credits diffusion of CO_2 from embryonic tissue for this decline. From day 15 to day 19, amniotic fluid pH then dramatically increases to nearly 8.0. Although the reason for this is unknown, Walker (1943) points out that the embryo selectively absorbs certain materials from the amnionic cavity during this time.

Tazawa (1971; 1972) and Tazawa et al. (1971b) measured the blood gas parameters of mixed venous and arteriolized chicken embryonic blood during development. Gas tensions rose from near atmospheric levels early in incubation to hypercapnic and hypoxic conditions late in incubation owing to the increased metabolic demand of the growing embryo. Diffusion of O_2 and CO_2 increases by a factor of 4.5 late in incubation because of increasing partial pressure differences between the internal and external environments. Embryonic acid-base status changes from respiratory alkalosis with partial metabolic compensation early in incubation to levels comparable to those of the adult hen late in incubation. By changing the incubation temperature, Tazawa (1972) showed that blood gas tensions were directly dependent upon the developmental stage, which is a function of metabolic rate.

Tazawa (1972) studied blood gas parameters on eggs with reduced respiratory area or increased diffusion capacity. Reducing the respiratory area resulted in severe hypoxia and respiratory acidosis. He considered this equivalent to hypoventilation in convective respiration. Tazawa (1972) also noted increased hematocrit and retarded embryonic development. He concluded that gas exchange over

the narrow and broad end of the egg is equivalent and performed in proportion to the total respiratory shell area. Until pulmonary respiration began, a respiratory advantage of the air space could not be considered to exist. Increasing the diffusion rate decreased pCO_2, promoted development, but did not significantly change hatchability. Those eggs which had both reduced respiratory area and increased diffusability showed decreased hatchability. Only pCO_2 is significantly affected by increasing the rate of diffusion across the shell because of its greater diffusion. Tazawa (1972) postulates that decreased hatchability in this case, and "death in the shell" in general is caused by O_2 deficit and not by CO_2 excess. Further, since he observed no difference in hatching time between controls and eggs with increased diffusion, Tazawa (1972) differs from Visschedijk's (1968c) conclusion that the pipping stimulus is caused by the gas tension levels in the air space and blood.

Dawes and Simkiss (1969) investigated the acid-base status of chick embryonic blood during incubation by measuring pH, pCO_2, HCO_3^-, and base excess (BE) levels. They found that pCO_2 rises continuously from about 20 mm Hg on day 11 to about 60 mm Hg on day 19. Generally, they found that blood pH varies between about 7.35 and 7.45 during days 11 through 21. Dawes and Simkiss (1969) incubated eggs at 39.4°C in a still air incubator, which, according to Romanoff (1972), causes approximately an 85 to 90% hatching mortality. As shown by Tazawa (1971; 1972) and Tazawa et al. (1971b) a substantial difference exists between arteriolized and mixed venous blood pH levels. The sample collection technique of Dawes

and Simkiss (1969) by heart puncture or decapitation does not seem to adquately discriminate for this difference and neither of these techniques could insure an anaerobic sample collection.

Dawes and Simkiss (1969) point out that increasing BE in the blood during incubation is probably due to increased HCO_3^- level. They feel that increases in the blood HCO_3^- come from the shell. They note that the chorioallantoic membrane first touches the shell on about the 10th day of incubation, reaches its maximum size on day 14, and functions from this time on to resorb mineral from the eggshell.

Dawes and Simkiss (1971) further elaborated on the natural respiratory acidosis occurring late in incubation by subjecting nine-day-old embryos to an artificial environment of 9% CO_2 in air. They documented a rapid increase in serum HCO_3^- which persisted throughout the remainder of incubation. Rejecting a renal mechanism as a source for extra HCO_3^-, they postulate eggshell resorption.

Dawes (1972) has attempted to measure O_2 tensions of the extraembryonic fluids. He collected and measured samples in atmospheric conditions and in a N_2 atmosphere. He found a significant difference in O_2 tensions between the two methods, indicating that either pO_2 was increased by exposure to air, or that pO_2 was decreased by O_2 lost from the sample to the N_2 atmosphere or both. The O_2 tensions of all extraembryonic fluids drop during incubation; there appears to be a gradient of O_2 richness decreasing from the outside to the center of the egg. Dawes suggests that perhaps gaseous exchange occurs across the skin of the embryo;

this accounts for greater acidity and lower pO_2 in the amniotic fluid (Walker 1943). He further correlates the low ebb of pO_2 in amniotic fluid on day 13 with the time that the total O_2 uptake by the isolated membranes reaches a maximum (Needham 1932 from Dawes 1972).

Dawes (1974) measured pH, pCO_2, and pO_2 on the extraembryonic fluids of the chick on days 12 and 15 after restricting gaseous exchange across the shell. He found a much greater resistance to death on day 12 than day 15 (mean time of 100% mortality 60-65 min day 15 and 105-110 min day 12). Dawes postulates that the low level of HCO_3^- in amniotic fluid results in a low buffering capacity towards fixed acids, and that with the loss of water and and increase in allantoic fluid uric acid, the buffering capacity of this fluid decreases from day 12 to day 15. Dawes suggested pCO_2 measurements of allantoic and amniotic fluids could be considered as convenient indices for assessing asphyxiation as a cause of "death in the shell".

Romanoff (1967) states that the metabolic rates of O_2 consumption are very high (400 to 100 cc O_2 per 100 mg dry wt) during the first five days of incubation, but from the 10th through the 20th day the rates drop from only 30 to 15 cc O_2 per 100 mg dry wt. Only a portion of O_2 uptake and CO_2 production is caused by the embryo; a significant fraction of metabolism is caused by the extraembryonic membranes or CO_2 evolution by egg albumen early in development.

Freeman (1962) noted an increase in O_2 consumption after the

onset of pulmonary respiration, and concluded that pulmonary respiration is determined by the 5 to 9% CO_2 level in the air space (Romijn and Roos 1938). Foreshadowing the work of Visschedijk (1968c), Freeman (1962) suggests that a high CO_2 level in the air space may be necessary to stimulate successful pipping, and that overventilation during the parafetal period may be the cause for death in the shell.

Beattie (1964) first accurately measured the gaseous exchange of individual chick embryos late in development. He found "that prior to pipping the embryo is subjected to extreme hypoxia and hypercapnia which must make the margin between death in the shell and survival very narrow. After pipping, the embryo relieves this situation by "hyperventilation"; excess CO_2 is excreted by raising the RQ and from CO_2 dissolved in the body surface moisture and membranes.

Tazawa et al. (1971a) found that blocking the respiratory area at either the blunt or the pointed end (about 0.25 of the egg surface) delayed embryonic growth at later stages of development and resulted in an increase in pre- and post-pipping mortality. They found that eggs coated with epoxy cement were significantly different from controls in that on days 12, 14, and 16 pO_2 was significantly lower. Tazawa et al. (1971a) note that these changes resemble the effect of hypoventilation in man.

MATERIALS AND METHODS

Animals

Chicken eggs were obtained from a commercial hatchery and quail eggs were obtained from the Florida Agricultural Experiment Station, Gainesville. Fertile White Leghorn and Bobwhite Quail eggs were incubated in a forced draft incubator (Petersime Model 4). Incubation temperature was 37.5°C and relative humidity was maintained at approximately 66%. Eggs free from cracks and defective shells were incubated within eight days after being laid and were automatically rotated every two hours. Unincubated eggs were stored at 20°C and were loosely covered to prevent excessive dehydration. Eggs were candled using a light source from a Bausch and Lomb dissecting microscope fitted with a cardboard screen around the lamp barrel to block scattered light.

Egg Exposure

Egg exposures were conducted in a 1 m^3 cubical chamber constructed of 0.5 cm plexiglass. The chamber's interior was accessible from a tightly fitted 27 x 54 cm door. A 250 cm^2 round window at the rear of the chamber could be opened to provide ventilation between exposures. The outer margins of both openings were sealed with weatherstripping. Lead wires and monitoring tubes entered the chamber by means of stopper-filled ports so that a sufficiently tight seal to the chamber was maintained.

Eggs were placed on a tray located in the center of the

chamber. The tray was an 18 x 40 cm wooden frame with a recessed 0.5 cm wire mesh floor. The tray stood 0.5 m above the floor of the chamber; it was coated with a polyurethane finish to prevent any interaction with the acidic exhaust cloud.

The chamber was heated before each exposure with a heat gun (Master Appliance Corporation HG 501) so that the temperature inside the chamber varied between 40°and 35°C during a 15 min exposure. Thermal stability was improved by encasing the exterior of the chamber with styrofoam sheeting. All exposures were made at incubation temperatures to maintain normal metabolic rates. During the initial minutes of an exposure, the chamber temperature was slightly higher than 37.5°C, while later in the exposure the temperature was usually several degrees below 37.5°C.

Solid rocket fuel (Vickers 1974), provided by the Jet Propulsion Laboratory, Pasadena, was ignited in a copper steambath bowl filled with sand located on the floor of the chamber. A 2 to 3 cm length of nichrome wire inserted through the fuel was connected to the leads of a lead-acid storage battery. The fuel was ignited by supplying electrical current to the nichrome wire until it heated to the fuel kindling temperature. During the fuel burn, the chamber was vented by removing a No. 4 stopper in the side of the chamber; this prevented excessive pressure buildup generated from the exhaust gases.

Mortality Studies

The comparative hatchability of chickens and quail exposed to the rocket fuel exhaust products was studied by varying the number

of exposures that eggs received and the concentrations of exposures. Based on preliminary study, six different weights of fuel were selected. These fuel quantities were predicted to yield gas concentrations which would range from "no effect" to lethality of the exposed embryos. Enough fuel to provide predicted values was weighed to an accuracy of $0.0001 \pm .003$ g as shown in Table 5 . Actual concentrations were continuously monitored during exposures by determining Cl^- concentration using the method of Madsen (1974); measurements are expressed as ppm of HCl. Preliminary exposure studies indicated that only 30 to 60% of the theoretical concentration of the exhaust gas could be assayed in the chamber atmosphere. Higher rates of recovery seemed to be positively correlated with increasing concentrations of gas in the chamber. This phenomenon probably existed in part owing to the adsorbtivity of exhaust gas components to the inner walls (Madsen 1974).

Treatment groups were exposed to exhaust gases one, two, three, or 14 times (as shown below). Each group received the same theoretical dosage for each exposure. Exposures were made at proportionately equal times of incubation for each species:

Number of exposures	Day(s) of incubation exposed	
	Chicken	Quail
1	19	20
2	12, 19	13, 20
3	4, 12, 19	4, 13, 20
14	4-18	4-18

Treatment groups consisted of 24 eggs (9 control and 15

Table 5. Theoretical and actual exhaust concentrations in a 1 m^3 chamber for various weights of solid rocket fuel. The expected exhaust concentrations were based on preliminary scrubber analyses (Madsen 1974).

Wt. of fuel g	Theoretical exhaust concentration ppm	Measured exhaust concentration ppm
0.1484	20	10
0.7420	100	50
1.4840	200	100
2.5970	350	175
3.7100	500	250
7.4200	1000	500

experimental) for chickens and 25 eggs (8 control and 17 experimental) for quail. Control eggs were exposed to 0 g of fuel, prior to the exposure of experimental eggs, under identical conditions. Each combination of exposure concentration and number of exposures (i.e. each treatment group) was triplicated. Hatchability of control eggs was normalized to 100% and the corresponding correction factor then applied to each treatment group. Hatchability as used here refers to the percentage of chicks that hatch from fertile eggs.

Weight Loss Determination

Eggs were weighed at the onset of incubation and subsequently every three days to the eighteenth day. Weights for chicken eggs were taken to the nearest 0.01 g on a Sartorius Model 1106 balance. Quail eggs were weighed at the onset of incubation and again on the twentieth day of incubation to the nearest 0.001 g on a Torbal Model ET-1 balance. Two days before hatching, eggs were individually placed in a partitioned box housed in the bottom of the incubator. After hatching, chicks were weighed and an approximate time of hatch was recorded ($\pm$ 8 hrs). The disposition of each egg was determined by candling or direct observation.

Blood Gas Analysis

The effects of solid fuel exhaust gases upon blood pO_2, pCO_2, and pH were measured on chick embryos during the twelfth day of incubation. Each treatment group consisted of eight eggs (3 control and 5 experimental). Based on mortality data obtained earlier, those combinations of numbers of treatments and doses (gas concentration

and temperature) which were predicted to most likely demonstrate a change in parameters were selected. Following exposure on day 12, the egg temperature was stabilized at 37.5°C in a walk-in incubator for at least 30 min before bleeding. During this time eggs were candled to locate the allantoic vein and to mark the shell for opening.

Blood samples were drawn into a 27 gauge needle which was placed on the barrel of a 1 cc syringe. A 100 µl micropipette had previously been placed inside the syringe barrel, with the outside diameter of the micropipette and the inside diameter of the syringe tightly sealed. The micropipette was installed so that it protruded 1 cm past the Luer end of the syringe. A small piece of Critoseal was wrapped around the protruding end of the 100 µl pipette, but the tip was left unobstructed. This further insured an airtight seal between the micropipette and syringe, and eliminated the dead air space in the Luer hub when the needle was installed. The proximal end of the micropipette was fitted with latex tubing which permitted a precisely metered oral vacuum. The needle and pipette were heparinized.

Blood samples were drawn from the allantoic vein. The opening, which was no larger than 1 x 1.5 cm, was cut in the shell with a Vibro engraving tool. Under a dissecting microscope, the membranes were lifted away from the opened area and with sharply pointed surgical forceps the allantoic vein was lifted to the level of the shell by hooking one arm of the forceps under the vessel. The needle was inserted into the vessel and up to 200 µl of blood

were taken anaerobically. As reported for a similar technique by Tazawa et al. (1971a), sampling time was usually less than two min from the time of removing the eggshell to completion of sample collection.

The blood sample was anaerobically transferred to a 100 µl capillary tube specially adapted in size for introduction into the sample port of the blood gas instrument. An Instrumentation Laboratories blood gas analyzer (IL Model 113) was used for measuring blood parameters. The pO_2 and pCO_2 were calibrated by slope standardization against known gas mixtures between each sample. The blood parameters of the sample were sequentially recorded within 1 to 2 min using the microtechnique described by IL for the instrument (1970).

Structural Damage

Elucidation of possible structural damage to the shell by exhaust gases was attempted following the method of Fujii (1974). The rationale of this experimentation was that changes in pore size or configuration, as observed by making negative casts of the air pores, would provide an index to shell damage.

Experimental and control halves of each egg were marked along the longitudinal axis. Control halves were covered with Parafilm (assuming that Parafilm was impervious to the exhaust gases) secured to the shell along the dividing mark with Scotchtape. An airtight seal was made by making a thin seam of white glue along both lateral borders of the tape (i.e. the Parafilm-tape and the tape-shell interfaces). Eggs were exposed once at measured concentrations of

175 and 500 ppm. They were then cut in half and the contents removed. The membranes were stripped from the shell and the shells were rinsed briefly in tap water. Organic matter was further removed by immersing the shell in 30% NaOH for 48 hrs. Shells were rinsed in tap water and air dried. The dried shell halves were filled with a thermal embedding resin normally used to embed tissues for transmission electron microscopy (Spurr Low-Viscosity Embedding Media, Polysciences, Inc.). The resin was cured for 8 hrs at 80°C.

The exterior of the shells were later marked with a grid and each grid box was labelled so that shell fragments from equivalent parts of the egg could be compared. The shells were then cut into small blocks with a bandsaw, labelled on the resin side, and immersed in 5% HNO_3 to dissolve the calcified shell. After the shell was dissolved, the cast was washed in water, coated with carbon black and gold, and examined with a Cambridge scanning electron microscope at the Kennedy Space Center using an accelerating voltage of 500 KV.

Statistical Methods

A trinomial regression was used to analyze the mortality data. The regression was accomplished with the aid of an IBM 360 computer and a standard "packaged" program (SPSS 6.07).

RESULTS AND DISCUSSION

Mortality Studies

The sensitivity of chicken and quail embryos exposed to SRM exhaust products is determined by examination of the treatments and hatchability for each treatment group. Three parameters are used to characterize sensitivity: the minimum lethal dose (MLD), the dose which kills 50% of the treated population (LD_{50}), and the minimum dose which causes 100% mortality of the treated population (extinction range). The MLD is usually described as the lowest range of dose values which cause death. Confidence limits for natural mortality must be determined so that any mortality which occurs beyond those limits can be ascribed to the treatment and not to other causes. In this study, after the control hatchability was normalized to 100%, the standard deviation (SD) of the normal mortality for control eggs was determined to be 10%. Any hatchability which occurs beyond a range of two SD (80 to 120%) can be attributed to the treatment with 95% confidence. The MLD, as used here is the range of dose concentrations which result in an 80% hatch. The LD_{50} is a single dose value determined from the data that is expected to kill 50% of the treated embryos. Confidence limits may or not be expressed with the LD_{50}; it is an indication of the SRM exhaust concentration necessary to kill embryos which are in the mid-range of sensitivity. The extinction range is the range of minimum gas concentrations which are predicted to be necessary to

kill all the embryos of a treatment group.

Chicken and quail treatment groups are categorized into those receiving one, two, three, or 14 treatments. Since the concentration of each exposure for those embryos that received multiple treatments were approximately the same (Appendix I), initial exposures are considered pretreatments and the final exposure concentration is used as a basis of comparison in studying hatchability differences between chickens and quail, and among different numbers of treatments.

For a given organism and category (i.e. chickens receiving one exposure) percent hatch (normalized) is plotted on the exhaust concentration of the final exposure. Figs. 1-6 show these results of the mortality studies. A trinomial regression determined from the experimental points is then employed to produce a predicted mortality curve for each organism and category.(SPSS 6.02).

The results of a single exposure on the mortality of chicken and quail embryos are shown in Figs. 1 and 2. For chickens, the LD_{50} calculated from the regression equation is 204 ppm (Fig. 1), and the MLD for a single exposure is 120 to 140 ppm (Table 6). Although no embryos survived exposures over 300 ppm, the predicted extinction range consists of concentrations over 350 ppm. Quail embryos are more sensitive than chick embryos to a single exposure (Fig. 2). The MLD is 100 to 130 ppm (Table 6), the LD_{50} is 175 ppm, and the extinction point is 350 ppm. Although the predicted hatch for both species is about 86% after a 100 ppm exposure, above this gas concentration quail hatch declines more rapidly than does chick.

Fig. 1 . Hatchability of chicken embryos receiving a 15 minute exposure to SRM exhausts on day 19 of incubation. Control hatchability is normalized to 100%.

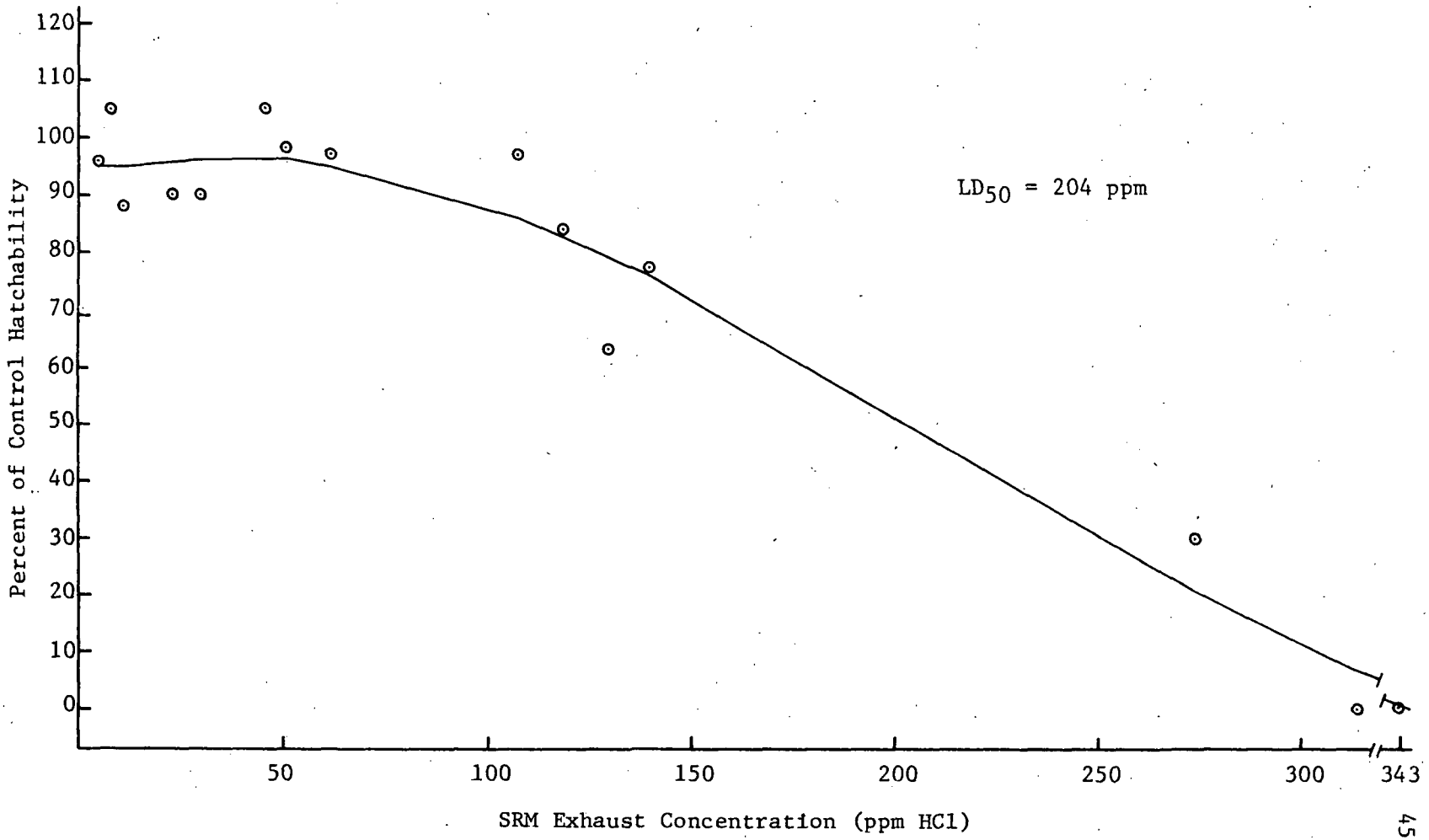


Fig. 2. Hatchability of quail embryos receiving a 15 minute exposure to SRM exhausts on day 19 of incubation. Control hatchability is normalized to 100%.

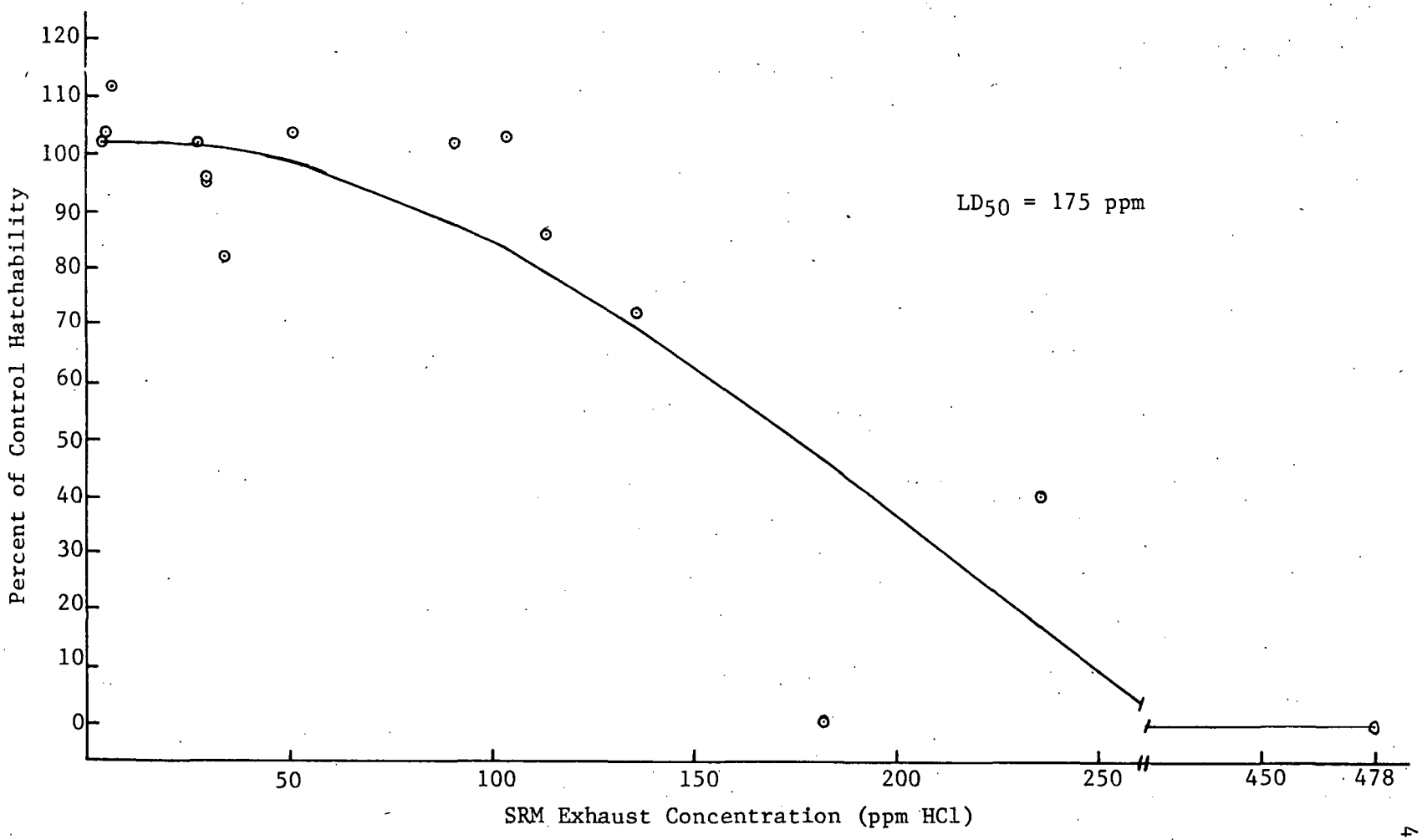


Table 6. The minimum lethal dose (MLD) range, the LD_{50}, and the extinction range (100% mortality) for chicken and quail embryos subjected to 15 min exposures to SRM exhausts on day 19 or 20 of incubation. The number of equivalent pretreatments is indicated.

Treatment	Group	MLD Exposure Concentration Range in ppm	LD_{50} ppm	Extinction Range ppm
No pre-treatment	Chicken Fig. 1	120-140	204	350
	Quail Fig. 2	100-130	175	350
One pre-treatment	Chicken Fig. 3	70-90	127	190-210
	Quail Fig. 4	40-60	86	160-180
Two pre-treatment	Chicken Fig. 5	30-50	75	180-200
	Quail Fig. 6	20-30	56	120-140

The effect of the final exposure on the mortality of chicken and quail embryos after one pretreatment is shown in Figs. 3 and 4. The MLD for chicken embryos is 70 to 90 ppm (Table 6); whereas, quail embryos appear to be more sensitive with an MLD of 40 to 60 ppm. The LD_{50} is predicted to be 127 ppm for chickens (Fig. 3) and 86 ppm for quail (Fig. 4). The extinction range for chickens is 190 to 210 ppm and 160 to 180 ppm for quail. Although the regression equations (Appendix II) predict hatchabilities less than 0% at the extinction range and increased hatchability at higher concentrations, graphically this has been modified so that once the hatchability is predicted to be 0% or less, that and higher concentrations are shown as 0% hatch. This artifact is caused by an inflection point of the 3d degree polynomial model.

The effect of the final exposure on the mortality of chicken and quail embryos after two pretreatments is illustrated in Figs. 5 and 6. For both species all concentrations of the final exposure seem to elicit a mortality response. The regression curve shows that the MLD for chickens is 30 to 50 ppm and for quail 20 to 30 ppm (Table 6). The LD_{50} for chickens is predicted to be 75 ppm (Fig. 5) and for quail is 56 ppm (Fig. 6). The extinction range for chickens is 180 to 200 ppm and it is 120 to 140 ppm for quail (Table 6).

Embryos show an increased sensitivity to the final exposure which is proportional to the number of equivalent preceeding exposures (Table 6). Table 6 shows that chicken eggs exposed once

Fig. 3 . Hatchability of chicken embryos receiving one pre-treatment and a 15 minute exposure to SRM exhausts on day 19 of incubation. Control hatchability is normalized to 100%.

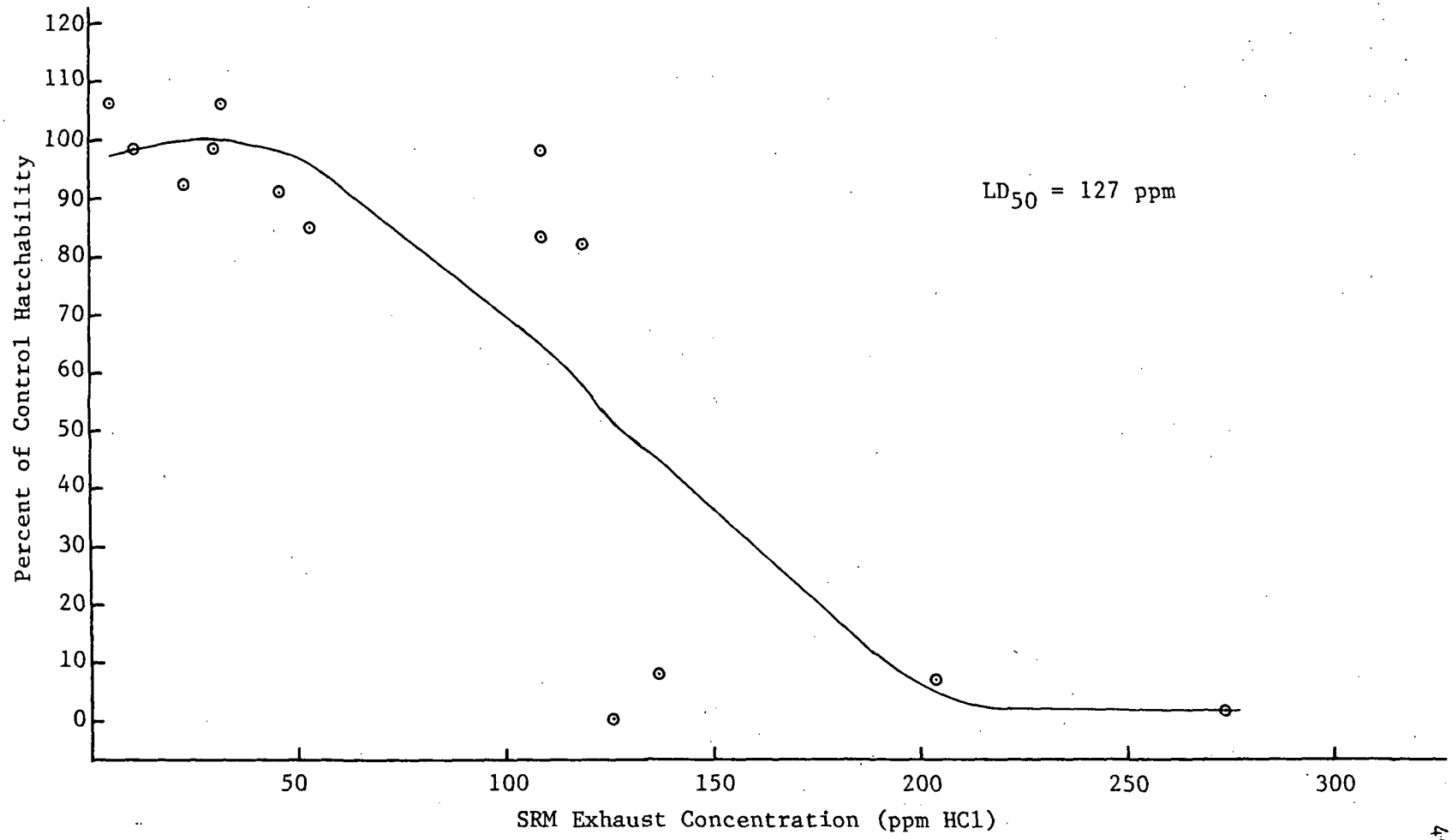


Fig. 4 . Hatchability of quail embryos receiving one pre-treatment and a 15 minute exposure to SRM exhaust on day 20 of incubation. Control hatchability is normalized to 100%.

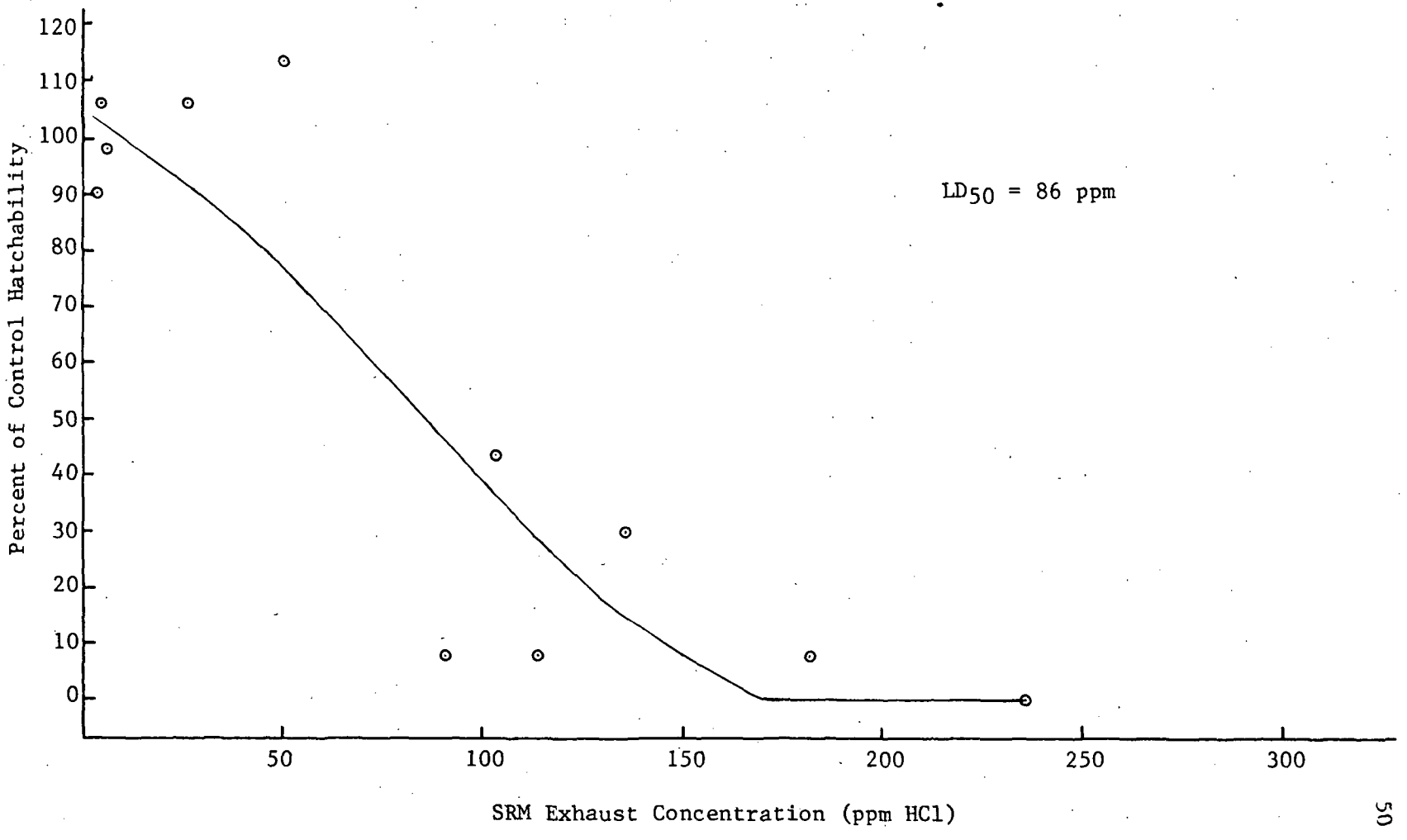


Fig. 5 . The hatchability of chicken embryos receiving two pre-treatments and a 15 minute exposure to SRM exhausts on day 19 of incubation. Control hatchability is normalized to 100%.

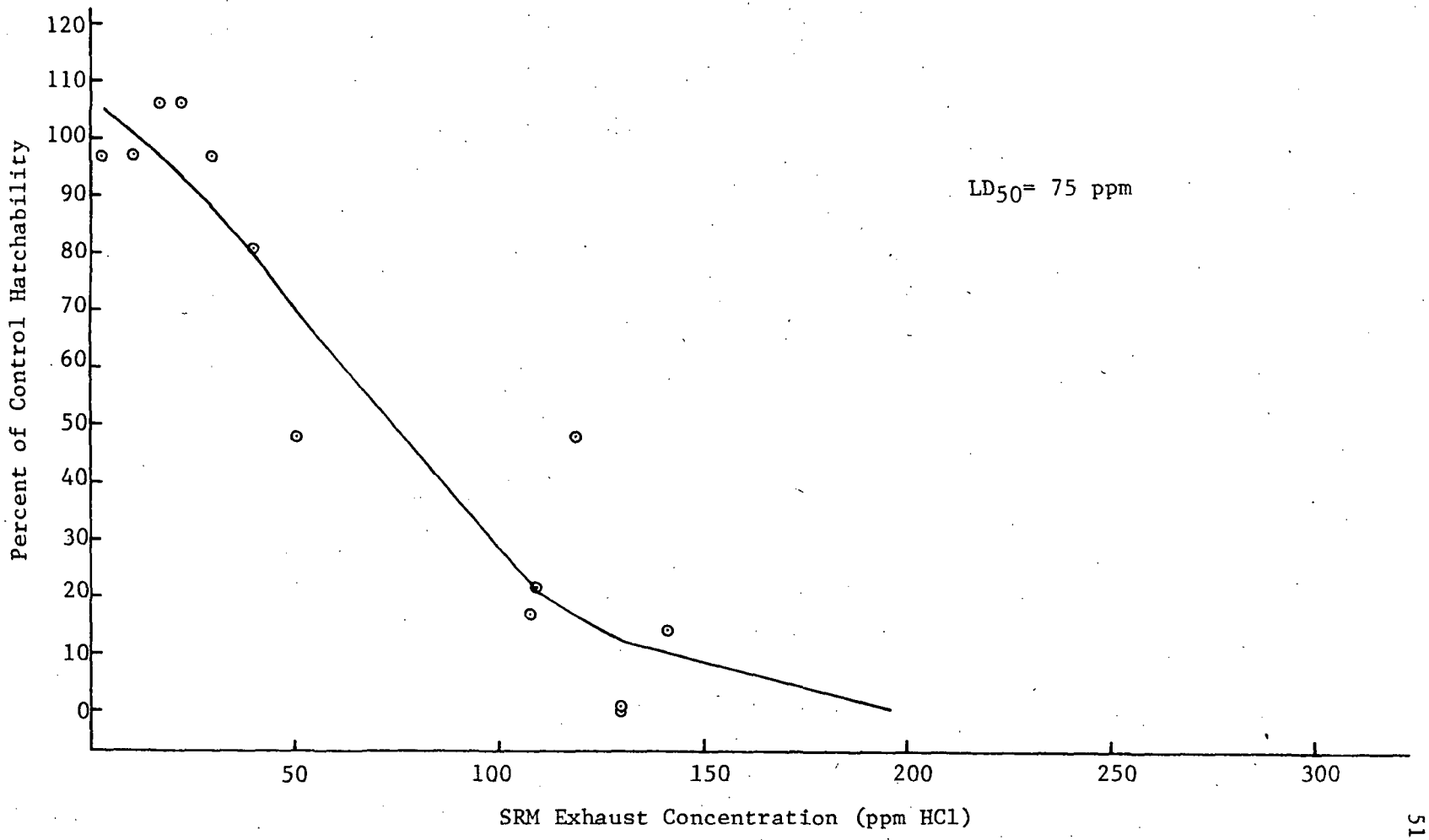
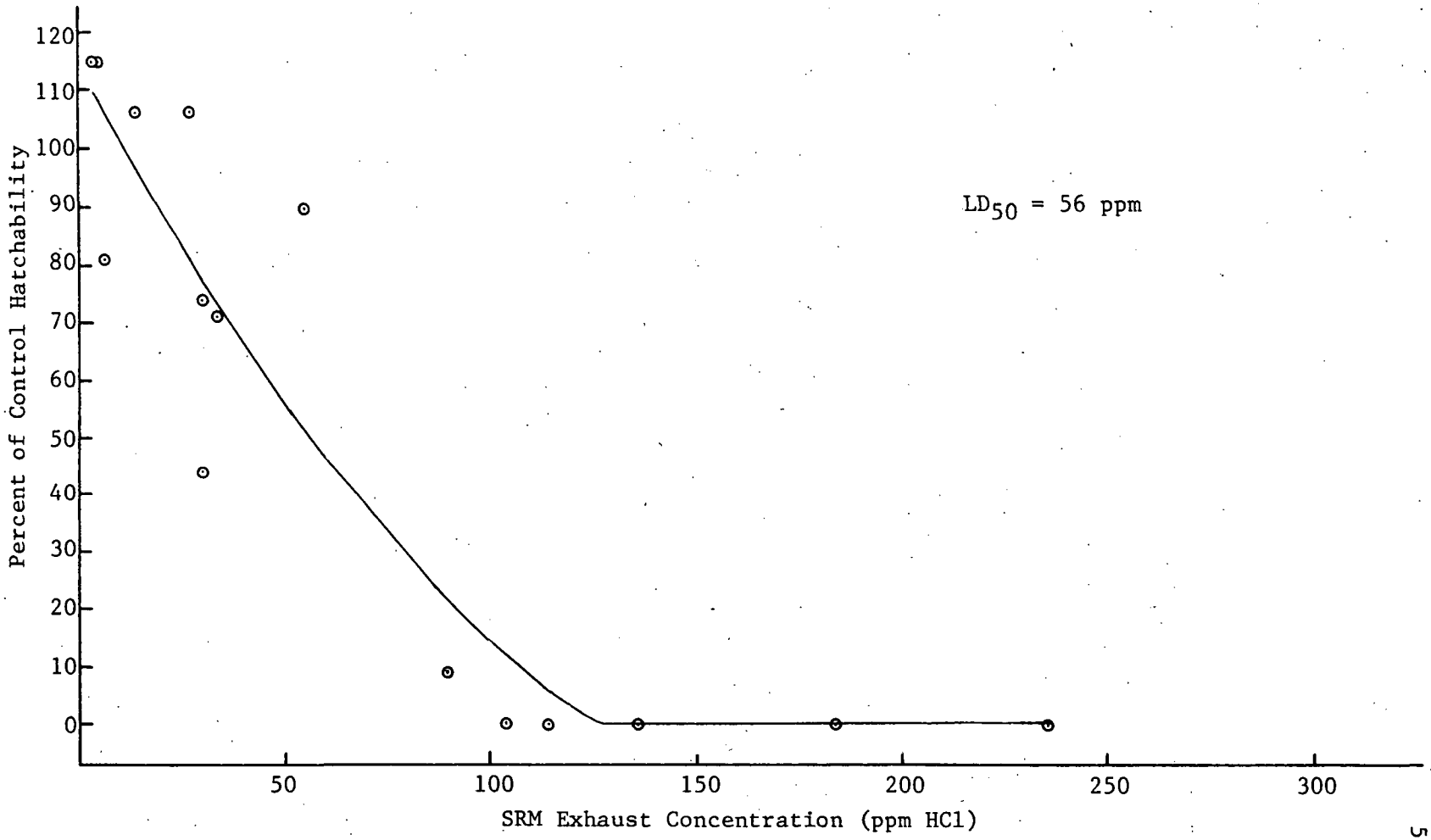


Fig. 6 . Hatchability of quail embryos receiving two pre-treatments, and a 15 minute exposure to SRM exhausts on day 20 of incubation. Control hatchability is normalized to 100%.



had an LD_{50} of 204 ppm (Fig. 1) while those receiving one pretreatment had an LD_{50} of 127 ppm for the last exposure (Fig. 3). This is a 38% reduction of the exhaust concentration needed during the final exposure to elicit the same LD_{50} response. Eggs which received two pretreatments had an LD_{50} of 75 ppm for the final exposure (Fig. 5). This is a further reduction of 41% in the LD_{50} fuel concentration. Likewise, quail embryos show approximately a 40% reduction in the LD_{50} concentration as the number of exposures are increased. Assuming that reduced hatchability can be extrapolated to more than two pretreatments, embryos which receive six equal exposures are projected to have an LD_{50} of 12 to 18 ppm for the final exposure (Fig. 7). Since increasing the number of exposures proportionately reduces the LD_{50} exposure concentration, this indicates that the effects of exposure are cumulative. Since multiple exposures reduce the LD_{50} exposure concentration by approximately 40% compared to one less exposures, sequential exposures are nearly additive in effect (a 50% reduction in LD_{50} concentration for each additional exposure would be additive). Confirmation that toxic effects can be cumulative and nearly additive are provided by Dawes and Simkiss (1971). They observed that exposure to 9% CO_2 on day 9 resulted in a rapid but incomplete compensatory rise in plasma HCO_3^- which remained for the duration of the incubation period. A similar rapid, but incomplete compensation to the lethal agents in SRM exhausts probably accompanies each exposure. The total time of exposure may contribute

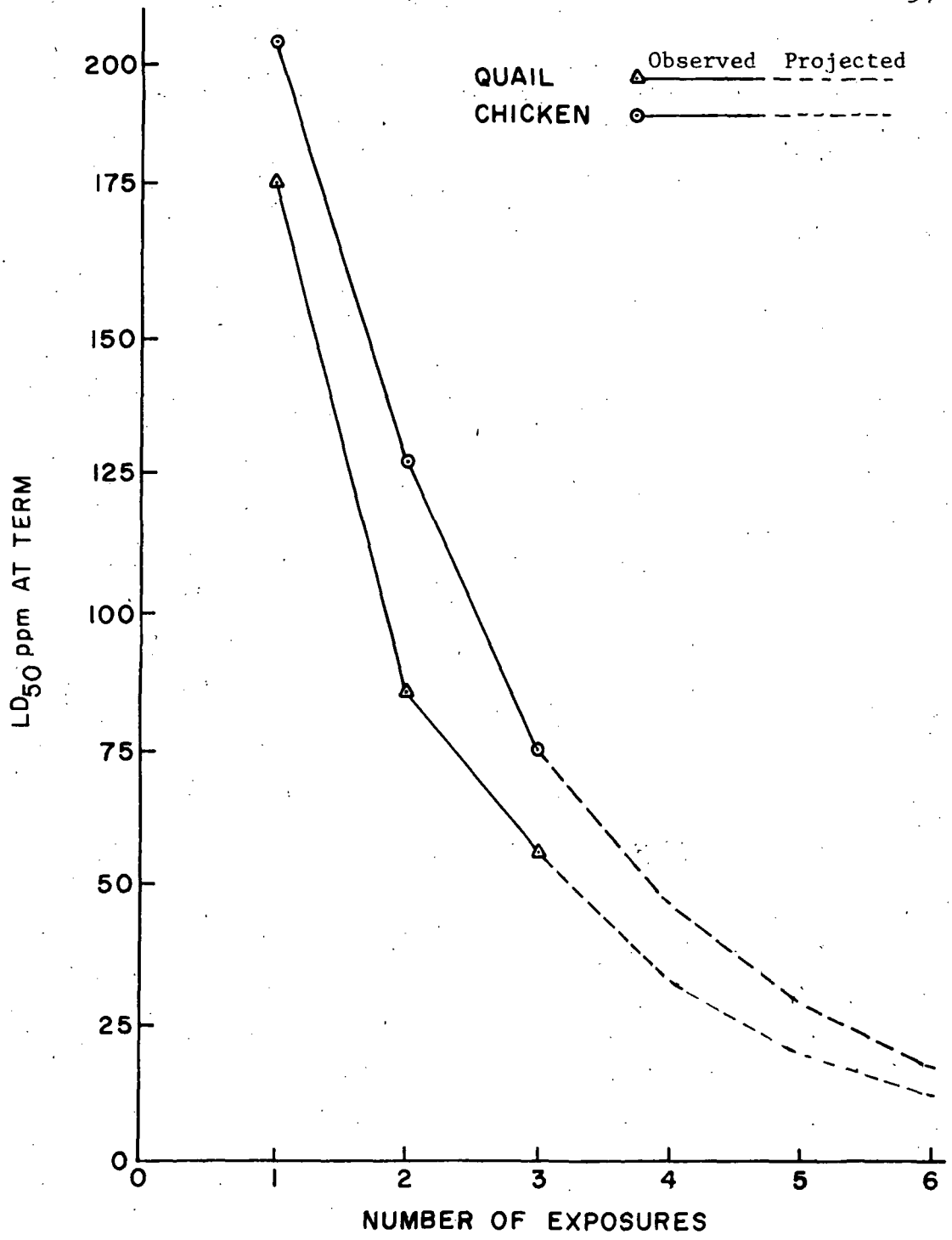


Fig. 7. Actual and projected LD_{50} for an exposure made before or during the parafetal period of chicken and quail based on the number of previous exposures.

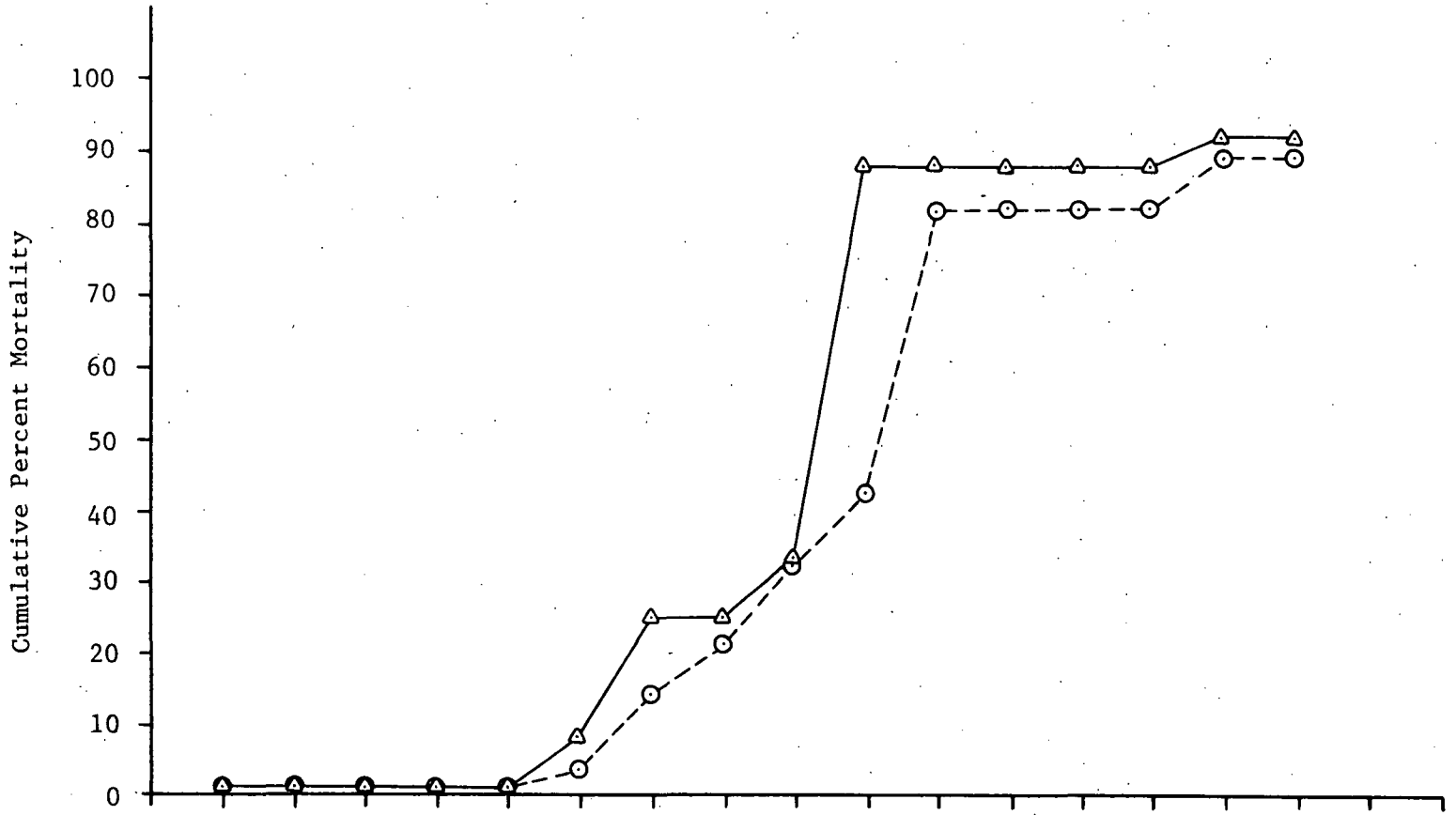
to lethal effects; unfortunately the experimental design used did not permit this question to be answered. For example, three 15 min exposures (45 min total time) on the same day to 100 ppm each may be more lethal than one 15 min exposure to 300 ppm on that day.

Effect of Chronic Exposure

Since repeated exposures apparently sensitize embryos to further exposure, two experiments were designed to determine the effects of daily exposure from days 4 to 19. The first experiment subjected 24 chickens (24 experimental and 5 control) and 28 quail (28 experimental and 9 control) embryos to 14 exposures ranging from 3.2 to 7.8 ppm. No chicken embryos died; one quail embryo died on day 18 and three died at hatching. Overall hatchability was 108% for chickens and 94% for quail embryos (normalized); the total concentration of the fourteen exposures was 95 ppm.

The second experiment, with the same sample size as the first, subjected chicken and quail embryos to SRM exhaust concentrations ranging from 11 to 34 ppm. Mortality effects in both species appeared after the total concentration (C_T) was 117 ppm (Fig. 8). This is consistent with the concentration which first causes lethality in a single exposure at 19 or 20 days (depending upon species), but is less than the concentration expected to be necessary to kill younger embryos. The greatest mortality for chickens occurred on day 10 (13 of 24 embryos died) and for quail on day 11 (11 of 28 embryos died). On day 10 the C_T was 200 ppm and on day 11 the C_T was 211 ppm. These are approximately the same

Fig. 8. Cumulative percent mortality of chicken and quail embryos subsequent to low level daily exposures to SRM exhausts. △——△ chicken ⊙---⊙ quail



Incubation day	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
Exposure number	1	2	3	4	5	6	7	8	9	10	11	12	13		14				
Exposure conc	34	14	14	14	18	21	17	34	19	13	11	16	20		16				
Exposure temp	37.7	36.6	37.	38.	33.7	37.7	37.1	35.5	35.6	38.	37.6	38.1	36.8		36.6				
Cum expose conc	34	50	64	78	96	117	134	168	187	200	211	227	247		263				

as the LD_{50} for a single exposure near the end of incubation. Cumulative mortality for chickens was 88% on day 10 and 82% for quail on day 11.

These data provide further evidence that the toxic effects of SRM exhausts are cumulative. Also, the MLD and LD_{50} levels of chronic exposure are comparable to embryos exposed once at the natural mortality peak near the end of incubation. Perhaps the resistance of the egg-embryo system is greater earlier in incubation and is offset by the debilitating effects of chronic exposure, thus making the mortality curves similar.

Chicken and quail embryos undergo stages in development during which natural mortality increases (Romanoff 1972). In chickens, the fourth, 11 to 13, and 19 to 20 days are the periods of greatest mortality. Of these, days 19 and 20 show the greatest mortality and days 11 to 13 the least. This is consistent with the observation that chicken embryos show their greatest sensitivity to SRM exhausts at 19 days (Appendix I). Although initial exposure on either day 4 or 12 did not show marked lethality effects compared with 19 day embryos, if embryos had been exposed only one time on these days, they probably would be sufficiently weakened so that many would expire at the natural mortality peake on day 19 (Appendix I).

Effect of Temperature

Temperature appears to significantly alter the lethal effects of SRM exposure. The effect of pretreatments masks temperature effects, but for embryos receiving one exposure on day 19 or 20, a trend is discernable (Table 7). Table 7 shows the difference between actual

Table 7. Percent deviation of actual hatch from predicted (from the regression curve) percent hatch for chicken and quail embryos exposed once to SRM exhausts grouped according to exposure temperatures.

Percent Deviation			
Exposure concentrations below the MLD or above the extinction range		Exposure concentrations in the effective lethal range	
Below 37.5°C	Above 37.5°C	Below 37.5°C	Above 37.5°C
+ 14	+ 10	+ 2	- 12
+ 4	+ 10	+ 2	+ 8
- 20	- 7	+ 11	- 47
- 5	+ 2	+ 6	
- 6	+ 2	+ 3	
+ 1	+ 12	+ 23	
- 4			
- 4			
+ 8			
+ 2			
$\bar{X}$ = - 1	$\bar{X}$ = + 5	$\bar{X}$ = + 8	$\bar{X}$ = - 17

and predicted mortalities for temperatures above and below the optimum of 37.5°C. As a control, variation from predicted mortality is shown for exposures that are outside the effective range of the exhaust. Table 7 indicates that when the mean exposure temperature is below 37.5°C, the hatchability is greater than expected. Conversely, high temperature reduces hatchability. The effect of high temperatures alone has been investigated by Romanoff (1972), who exposed 13 day chicken embryos to 55°C for 30 min and found no mortality; likewise ringnecked pheasant (Phasianus torquatus) eggs heated to 50°C showed no mortality when exposed for 70 min. This evidence indicates that decreased hatchability observed after exposure to SRM exhausts and elevated temperature results from synergism between exhaust and temperature; high temperature alone has no lethal effect and SRM alone has a reduced effect. Thus, exposures which are below the MLD or above the extinction range should show random variation of actual mortality about the predicted curve. The data in Table 7 substantiate this since mortality associated with exposure at low temperature is equally distributed about the predicted curve, and exposures at high temperature average 5% more than the predicted hatchability. Eggs exposed to concentrations in the effective lethal range showed greater hatchability than predicted at low temperatures and lower hatchability than predicted at high temperatures.

The effect of temperature is probably a physical phenomenon. An egg at 37.5°C placed into a 39°C environment could serve as a

cool point of condensation. This condensation would provide an attraction for the hydroscopic HCl fraction and other gaseous fractions of the exhaust, thus intensifying the effect of exposure. Conversely, an egg warmer than its environment would have some protection against lethal effects of exposure.

Effect on Water Relations

Rate of water loss from avian eggs is constant throughout incubation (Ar et al. 1974); a deviation from this loss rate can be determined by weighing the egg at frequent intervals. Since eggs lose 18% of the original egg weight during incubation, it is germane to express the eggshell permeability $\dot{M}_{H_2O}$ (i.e. weight loss in mg per day) as a percentage of the incubation weight of the egg. Further, since eggs were weighed at unequal intervals, the percent $\dot{M}_{H_2O}$ is more meaningfully expressed as the percent weight loss in mg per hr. Thus, the percent rate of water loss is here defined as:

$$\% \dot{M}_{H_2O} = W_\Delta \cdot T^{-1} \cdot W_I^{-1}$$

where: $\% \dot{M}_{H_2O}$ is the percent rate of water loss per hour.

W_Δ is the change in weight of the egg in a time interval

T is the time in hrs between successive weighings.

W_I is the original incubation weight of the egg.

Incubating fertile chicken eggs in a dessicator is a means of artificially inducing a change in the percent $\dot{M}_{H_2O}$. Fig. 9 demonstrates that dessicated eggs dramatically increase in the percent $\dot{M}_{H_2O}$ to a level nearly three times that normally observed.

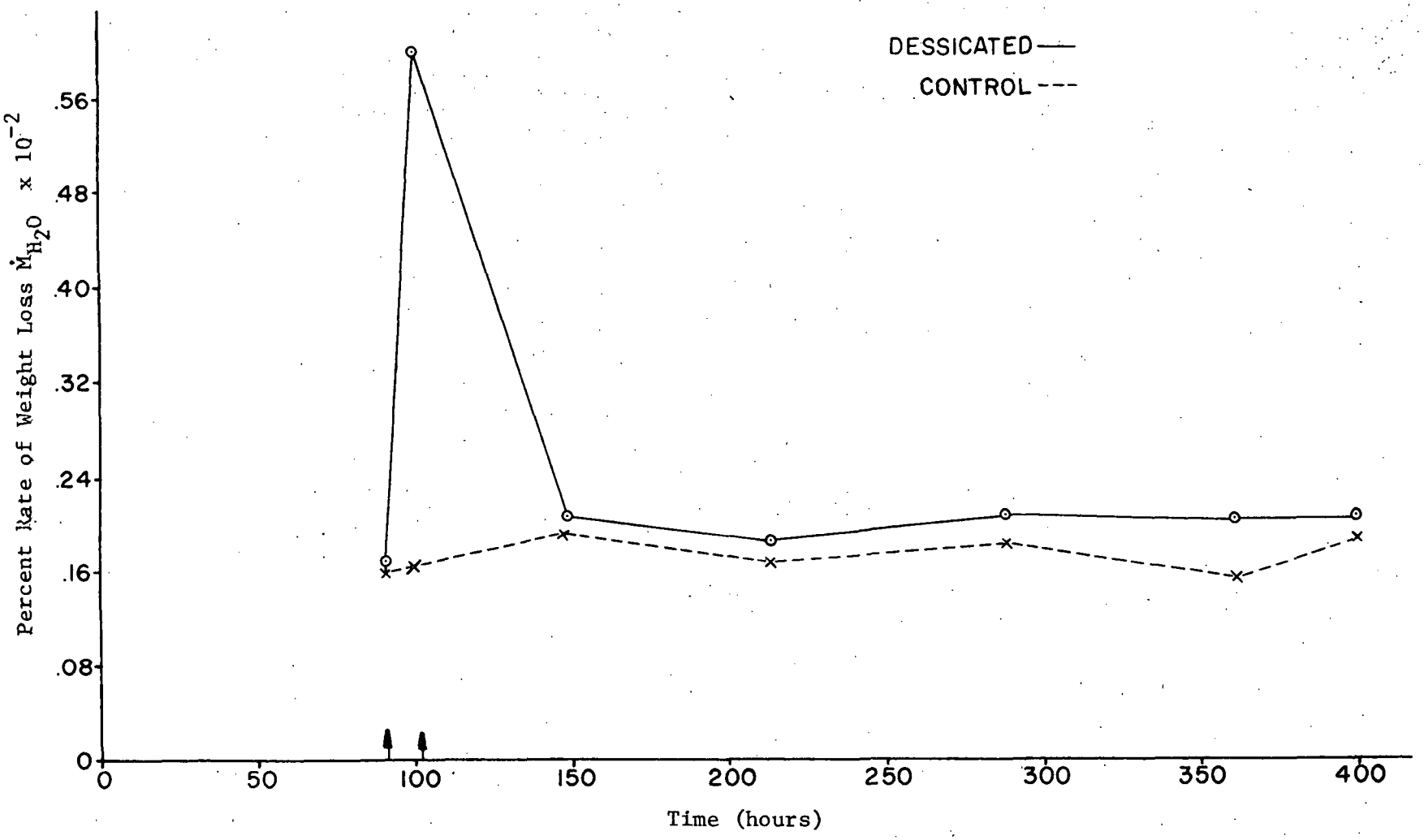


Fig. 9. The effect of incubation in a dessicator for six hours on the percent $\dot{M}_{H_2O}$ after 94 hours of incubation. Arrows indicate the initiation and termination of dessicator incubation.

Change in the rate of water loss of experimental eggs which received two successive exposures of 262 ppm (after 108 hrs of incubation) and 274 ppm (after 298 hrs of incubation) is shown in Fig. 10. In the interval after the initial exposure (108 to 133 hrs of incubation) the percent $\dot{M}_{H_2O}$ increased by 2.5 times. After the second exposure the percent $\dot{M}_{H_2O}$ for the experimental eggs again more than doubled, and the elevated rate was maintained from 300 to 366 hrs. Control eggs maintained a relatively constant percent $\dot{M}_{H_2O}$, with most of the observable flux probably due to experimental error in weighing. Eggs were not weighed for 25 hrs after the first exposure. This is significant because the change in the percent $\dot{M}_{H_2O}$ is assumed to have occurred as a result of exposure and within the first hour after the initial weighing. The percent $\dot{M}_{H_2O}$, for the interval 108 to 133 hrs, is a mean for the entire interval. After exposure, eggs were immediately returned to the incubator. Thus, for approximately 99% of the time interval measured, the treated eggs were re-establishing the homeostatic condition of the control eggs. The physiological disruption of water loss during a 15 min exposure must be extreme to cause the mean rate for the entire measured 25 hr period to be elevated to a level nearly three times that of the control level.

Fig. 11 shows the effect of an exposure to 189 ppm. The eggs were weighed eight hrs after exposure. Since the percent $\dot{M}_{H_2O}$ is averaged over an eight hour period, it increased

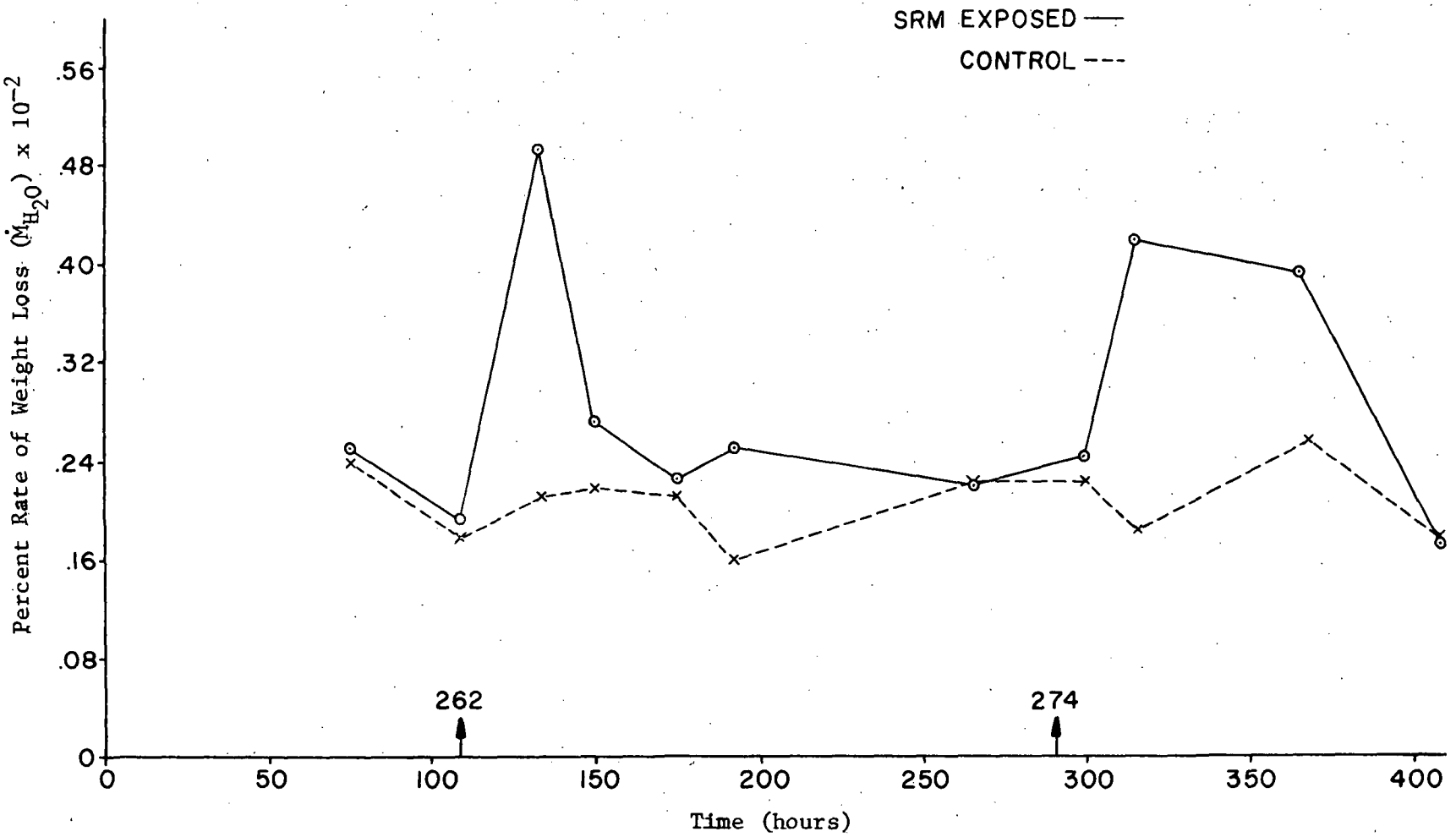


Fig. 10. The effect of exposure of fertile chicken eggs to SRM exhausts on the percent of weight loss ($\dot{M}_{H_2O}$). Experimental eggs received a 15 minute exposure to 262 ppm after 108 hours of incubation, and to 274 ppm after 298 hours of incubation.

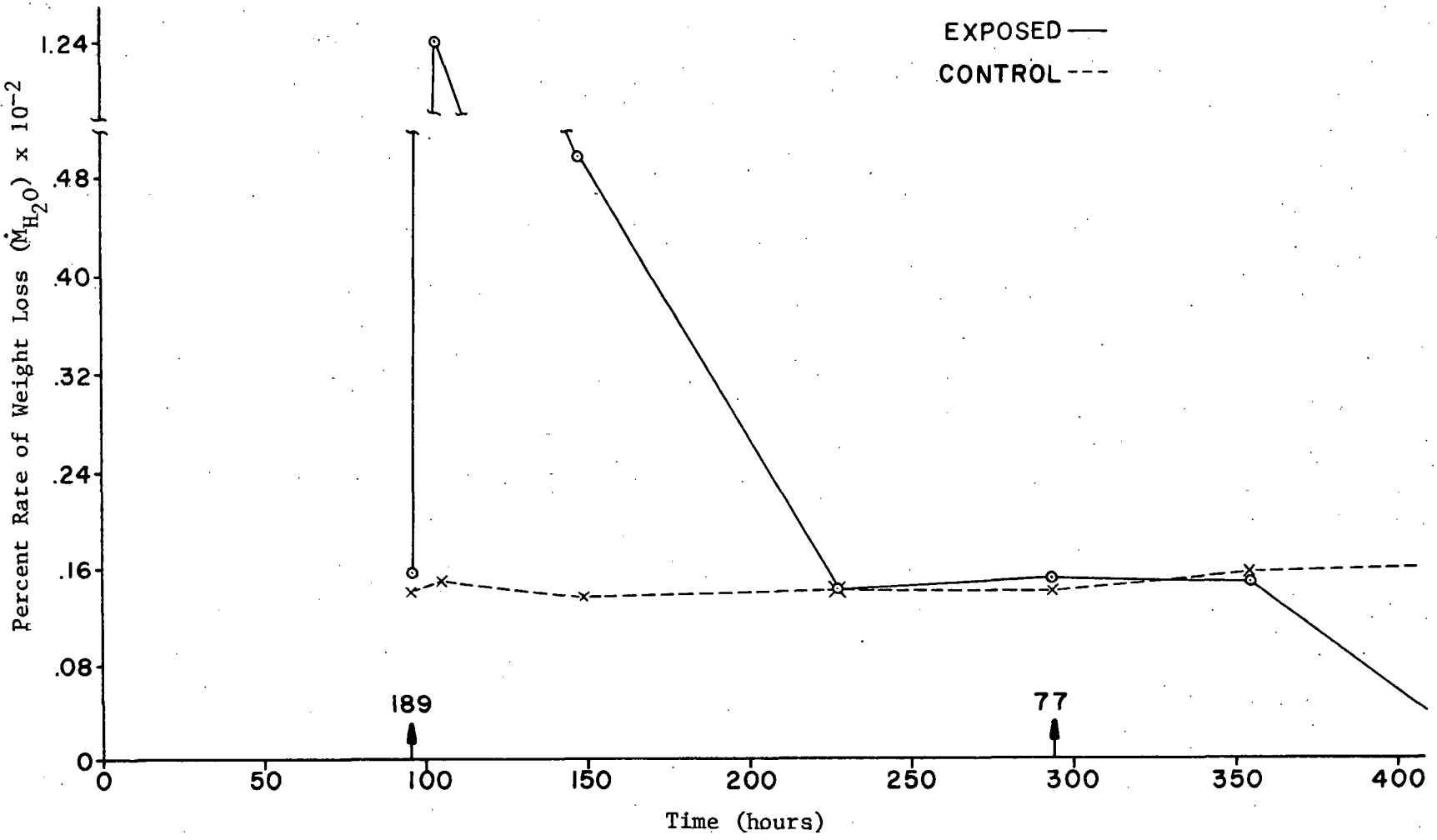


Fig. 11. The effect of exposure of fertile chicken eggs to SRM exhausts on the percent of weight loss ($\dot{M}_{H_2O}$). Experimental eggs received a 15 minute exposure to 189 ppm after 105 hours of incubation, and to 77 ppm after 289 hours of incubation.

by a factor of 5 to 8. This observation indicates that the extreme disruption of the percent $\dot{M}_{H_2O}$ during and immediately following exposure to SRM exhaust. A later exposure of 77 ppm is not reflected in a change in the percent $\dot{M}_{H_2O}$, probably because of a smaller exposure concentration and the extremely long interval (71 hrs) over which the percent $\dot{M}_{H_2O}$ is averaged.

Fig. 12 shows a treatment group exposed three times. Exposure to a concentration of 314 ppm when the eggs were incubated, and to 443 ppm after 171 hrs of incubation elicited predictable responses in the percent $\dot{M}_{H_2O}$. However, after 295 hrs of incubation, the percent $\dot{M}_{H_2O}$ dropped to zero. No further weight change was observed even though eggs were exposed to 531 ppm on the 14th day of incubation. An explanation for a lack of further weight loss may be as follows.

After 295 hrs the eggs in this treatment group had lost an average of 6.1 g (9.5% of their incubation weight). This was 1.64 times the weight loss of controls in the same time interval, and 295 hrs was 0.596 of the total mean time to hatch. Since water loss is linear throughout incubation, the control eggs would lose 1.68 times the amount of water they had lost up till 295 hrs by the time they hatched. By 295 hrs, the experimental eggs had lost the same amount of water that would be predicted for them to lose throughout the entire course of incubation. Perhaps when this finite amount of weight loss is attained, remaining water may be bound to the membranes and dense albumen in such a manner that

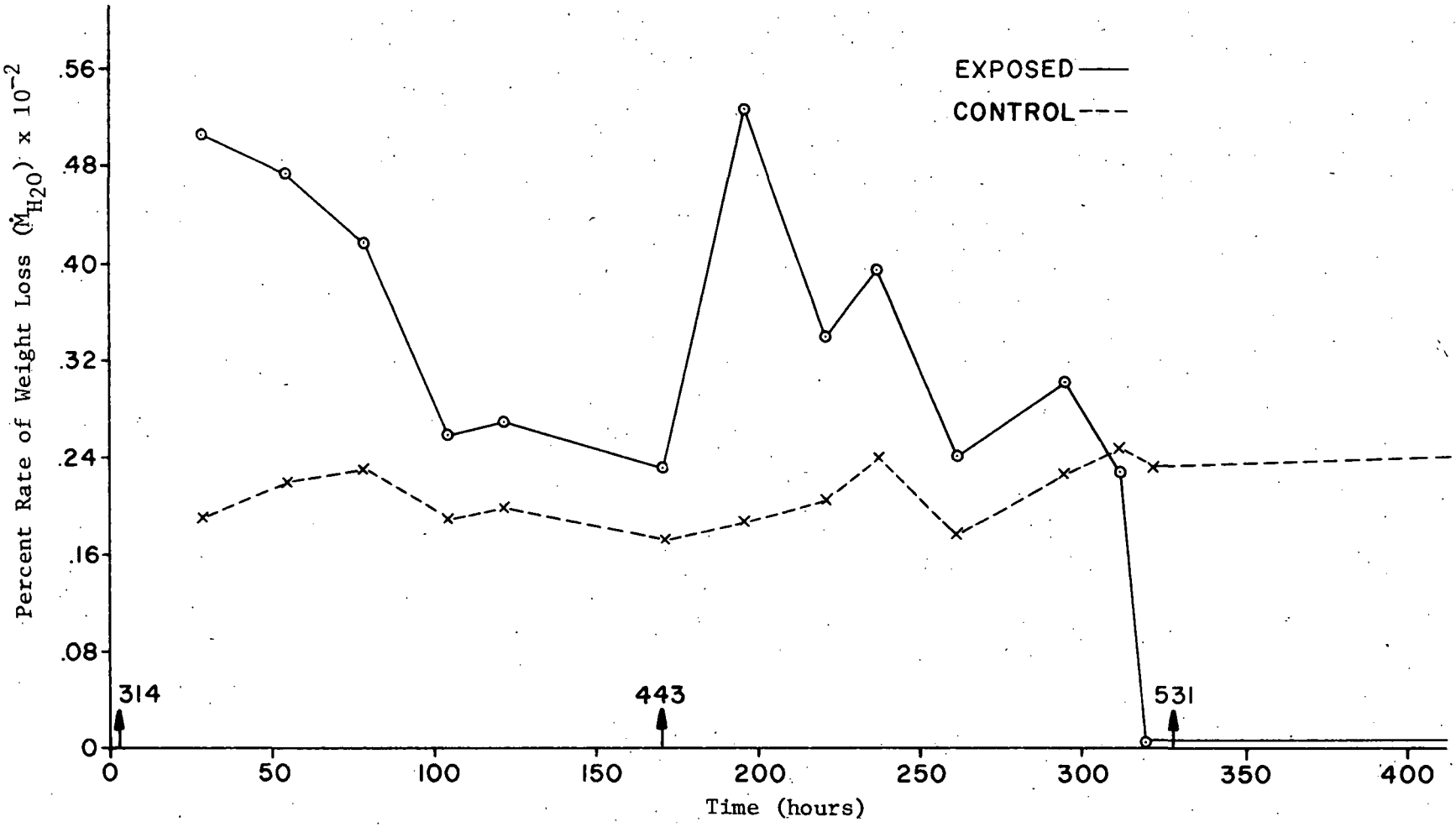


Fig. 12. The effect of exposure of fertile chicken eggs to SRM exhausts on the percent of weight loss ($\dot{M}_{H_2O}$), Experimental eggs received a 15 minute exposure to 314 ppm prior to incubation, 443 ppm after 171 hours of incubation, and 531 ppm after 320 hours of incubation.

further weight loss is not immediately possible.

To understand the mechanism by which SRM exhausts dehydrate the egg, and thus, contribute to mortality, a review of normal water regulation is appropriate. Marshall and Cruickshank (1938) suggest that air pores may develop around protein strands that pass from the deep layer of the outer cuticle covering to the outer lining membrane of the shell before calcification occurs. Although the surface pore structure varies with different species (Becking 1975), Marshall and Cruickshank (1938) have shown that the surface plaque (the head of the mushroom produced by a negative cast of the air pore), which is filled with an organic, proteinaceous, sulfur-containing material (Gilbert 1971), is essential to proper water relations and respiratory gas exchange. This organic material is hydroscopic, and by a capillary action functions to draw water from the albumen to the increased evaporating surface of the plaque. Natural protein filled pores have been shown to evaporate water forty times faster from albumen than those from which the organic material has been removed (Marshall and Cruickshank 1938). This function probably begins by the fourth day of incubation (Wolken and Schwertz 1948) when the initially hydrated shell membranes and shell pores become gas-filled to permit the embryo to begin effective allantoic respiration (Wangensteen et al. 1970/1971; Wangensteen and Rahn 1970/1971). The shell pores then assume the function of regulating water loss during the remainder of incubation (Wangensteen and Rahn 1970/1971). In a review of the effect of the rate

of water loss on hatchability, Landauer (1960) cites numerous studies which show that hatchability is correlated with the rate of water loss during incubation. Wolken and Schwertz (1948) suggest that during embryonic development the rate of gas influx is controlled to some extent by the moisture content of the shell membrane.

The mechanism by which SRM exhausts cause mortality is hypothesized, in part, from the weight loss results (Figs. 10-12). The SRM exhaust is very hydroscopic (Nimmo et al. 1974; Rhein 1973) and the percent $\dot{M}_{H_2O}$ from two 15 min exposures has been shown to be able to cause water loss from the egg during the first 12 days of incubation that is equal to that usually lost during the entire incubation (Fig. 12). After exposures to 150 ppm or greater concentrations, eggs accumulated large amounts of surface moisture, probably drawn from within the egg. This surface moisture appeared when the egg was removed from the exposure chamber to the more humid atmosphere of the room. Even though the egg was warmer than the surrounding air, the egg accumulated moisture, indicating that moisture was drawn from within the egg. This qualitative observation corroborates weight loss observations concerning the extreme and rapid dehydration which accompanies exposure. Based on Romijn's work (1950), drying of the shell pores and membranes during exposure would facilitate diffusion of the SRM exhausts into the egg. This may account in part for the increased sensitivity of pretreated eggs.

Effect on Blood Parameters

The results of study on various blood parameters of 12-day-old chicken embryos subsequent to SRM exhaust exposure(s) are given in Table 8 and in Appendix III. Generally, values obtained for control eggs are comparable to those obtained by Tazawa et al. (1971b); however, differences exist in two parameters which are worthy of mention. The mean chick weight seems to be significantly different. The value recorded in this study was 5.22 g; Tazawa et al. (1971b) reported 4.44 g for 12-day-old embryos. This difference is probably due to breed (White Leghorn vs White Rock) or incubation conditions. The other difference occurs in the values for pCO_2. The result published by Tazawa et al. (1971b) of 18.1 ± 2.6 for a 13 day chick weighing 5.94 g is in closer agreement to the value measured in this study of 18.0 ± 1.4. Tazawa et al. (1971b) and others (Romanoff 1960; Romijn and Roos 1938) have documented the daily increase in pCO_2. All blood parameters measured by Tazawa et al. (1971b) were regressed against embryo weight and in this light the pCO_2 discrepancy between our results is not as great as it initially appears.

Regardless of the number of exposures or concentrations of those exposures, the pO_2 appears to remain at a fairly constant level (Table 8).

Chick weight up to day 12 did not appear to be affected by exposures made earlier in incubation (Table 8).

Eggs receiving one exposure on day 12 displayed a decreasing

Table 8. Embryonic weight and blood gas properties of arterialized blood of 12 day embryos which were exposed to various numbers of exposures and concentrations of SRM exhausts.

Number of exposures	Group	N	C_T ppm	Individual exposures ppm	Exposed on days	P_{O_2}	P_{CO_2}	pH	BE	HCO_3^-	T_{CO_2}	Weight g
0	Control	24	0	0	-	89.9 ±4.9	18.0 ±1.4	7.630 ±.045	-3.7	18.6	18.8	5.218 ±.544
0	Tazawa et al. 1971a	20	0	0	-	79.8 ±3.1	14.0 ±2.1	7.623 ±.034	-5.9	14.5	14.8	4.44 ±.58
1	1	5	22	22	12	90.6	18.5 ±1.4	7.626 ±.018	-3.3	19.0	19.2	5.364 ±.198
1	2	6	44	44	12	91.1 ±3.5	18.5 ±1.1	7.637 ±.024	-3.3	19.0	19.2	4.530 ±.284
1	3	5	152	152	12	90.9 ±4.7	22.2 ±3.1	7.568 ±.041	-3.1	19.7	20.0	4.852 ±.390
1	4	5	233	233	12	95.2 ±3.8	26.1 ±3.2	7.533 ±.077	-1.5	21.5	22.0	4.124 ±.406
1	5	5	322	322	12	88.0 ±0.8	30.0 ±2.1	7.529 ±.038	+1.7	24.6	25.2	4.445 ±.270
2	6	5	104	68,36	4,12	84.6 ±4.3	21.5 ±2.6	7.641 ±.025	-1.7	20.6	20.8	4.964 ±.381
2	7	5	194	89,105	4,12	90.1 ±4.0	18.7 ±0.9	7.654 ±.051	-1.8	20.6	20.7	5.331 ±.453
2	8	5	216	138,78	4,12	86.9 ±4.9	19.0 ±0.8	7.616 ±.030	-3.3	19.0	19.3	4.944 ±.221
3	9	5	88	24,36,29	4,8,12	91.6 ±2.4	20.2 ±1.6	7.661 ±.051	+0.6	22.8	22.9	5.034 ±.341
6	10	5	189	16,39,24 34,40,36	4,6,8 10,11,12	90.9 ±2.5	21.7 ±3.1	7.510 ±.075	-6.3	16.9	17.3	5.239 ±.661

Values are means ± S.D.

pH at higher exposure levels (Table 8). This was accompanied by a concommitant rise in pCO_2. Since rise in pCO_2 and HCO_3^- usually result from drop in pH (Demers and Sahlad 1973), this change in the embryo probably occurred to compensate for falling pH. Base excess (BE in meq/l), bicarbonate concentration (HCO_3^- in meq/l), and total CO_2 content (T_{CO_2} in mM/l) were calculated from the mean pH and pCO_2 from each treatment group using the Siggaard-Anderson Base Alignment Nomogram (Siggaard-Anderson 1963). Base excess increased with exposure to increasing concentrations of the SRM exhaust cloud.

Eggs exposed two to three times did not exhibit a trend with any parameter (Table 8). This was probably because the cumulative effect of early exposures, the concentration of the final exposure, or the stage of embryonic development did not permit effects to be shown.

Those eggs which received six exposures showed a decrease in pH and a slightly elevated pCO_2 (Table 8). These factors combined to yield a BE of -6.3. This result differs from those above, which suggests that these embryos were compensating to a chronic exposure.

This author postulates that initially carboxyhemoglobin increases, which tends to cause respiratory acidosis. This, combined with the extreme acidity of the exhaust cloud further lowers the buffering capacity of the blood, and a depression of the blood pH is initiated. Compensation is made by a rapid accumulation of HCO_3^- from various sources such as the shell and RBC's to buffer against the drop in pH. Additional buffering capacity is offered by hemoglobin and serum proteins, and a subsequent increase

in H_2CO_3, and dissolved and bound CO_2 will occur. Mammalian systems would attempt to equilibrate this acidemia by hyperventilation, so that excess CO_2 can be eliminated. The alteration of blood gas parameters observed here is very similar to

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 \text{ solution} \rightleftharpoons CO_2\uparrow$$

that postulated by Dawes and Simkiss (1971) for chick embryos subjected to respiratory acidosis and to the natural respiratory acidosis observed by Tazawa et al. (1971ab) during incubation. Since the chick embryo is incapable of hyperventilation, one might expect that Cl^- would be exchanged with the RBCs and that renal excretion of HCO_3^- would occur. However, concurrent with H^+ addition from the exhaust gases is the addition of Cl^- (the HCl component). Exogenously increased serum Cl^- would prevent any substantive chloride shift from occurring. In compensation to this anion, one may expect some cellular contribution of K^+, making the serum hyperkatrimic. CO absorbed will bind hemoglobin and reduce its buffering capacity against HCO_3^-. Dramatic dehydration of the egg system should further increase serum hypertonicity; further, the hydroscopicity of the exhaust gases which cause this water loss may provide a driving force for rapid diffusion of the hydroscopic fractions of the exhaust gases into the relatively hydrated internal milieau of the egg.

Exposure of avian embryos to SRM exhausts seems to be more lethal than exposure to individual exhaust components (Baker and Tumasonis 1972; McGrath and Moffa 1972). This synergism probably

results from combined action of the components on reducing serum buffering capacity, causing dehydration, and inducing respiratory acidosis.

Structural Damage Analysis

All attempts failed in producing casts to determine if exposure to SRM exhaust caused structural damage to the shell. Either the embedding fluid was too thick or organic material remained in the air pore . Scanning electron microphotographs (Fig. 13) revealed evidence of the base of air pores, but the "mushroom" expected of good quality casts was absent.

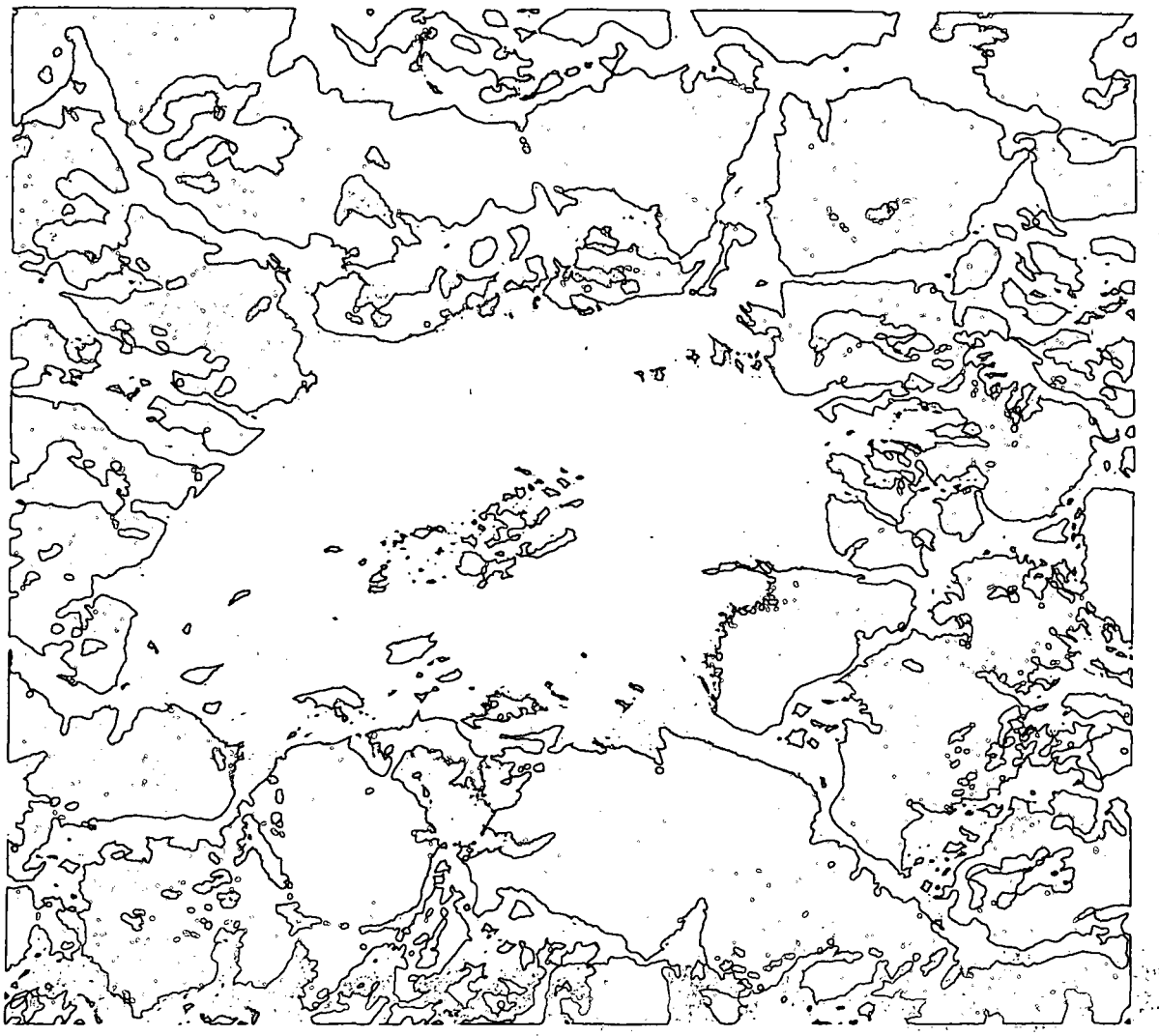


Fig. 13. Scanning electron photomicrograph of a negative cast showing the basal portion of an air pore. The chicken egg was exposed to 500 ppm SRM exhausts. x 400.

SUMMARY

1. Chicken embryos exposed to SRM exhausts for 15 min on day 19 demonstrated an LD_{50} of 204 ppm. The LD_{50} for quail on day 20 was 175 ppm.
2. Additional exposures (pretreatments) made earlier in incubation decreased the LD_{50} of the final exposure by approximately 40% for each additional exposure. Embryos exposed to a small daily concentration had an MLC of 117 ppm and an LD_{50} of approximately 200 ppm by day 14 for cumulative exposure concentrations.
3. Quail embryos appeared to be more sensitive to exposure than chicken embryos, probably owing to the larger surface area to volume ratio of the egg.
4. LD_{50} values suggest that SRM exhausts may have a synergistic lethal effect when compared to components of the exhausts
5. SRM exhausts cause extreme dehydration of avian eggs. The rate of water loss from chicken eggs measured during an eight hour period increased five times because of a 15 min exposure.
6. Blood gas analyses on 12-day-old embryos showed decreased pH at cumulative exposure concentrations greater than 200 ppm. Carbon monoxide in the exhausts probably increased carboxyhemoglobin, reducing buffering capacity. This acidosis was partially compensated by increased HCO_3^-; pO_2 was not affected. Exogenously derived Cl^-, plus increased HCO_3^- may shift intracellular K^+, making the serum hyperkatremic.

APPENDIX I

Percent hatchability and mortality of chicken embryos exposed to SRM exhausts.

Treatment Group	Group Number	N	Percent Hatch Control= 100%	C_T ppm	Temp °C	Observed Mortality
Chickens	3	14	95	5	22.0	4
1	23	13	105	8	38.4	0
exposure	41	12	88	11	38.6	2
	45	14	90	23	37.4	2
	27	14	90	30	37.3	2
	5	13	105	46	35.9	0
	48	14	98	51	39.0	1
	8	13	97	62	38.3	1
	33	13	97	109	37.9	1
	19	15	84	119	36.6	3
	38	15	63	130	39.1	6
	30	15	77	140	36.3	4
	20[a]	13	97	141	34.9	1
	11	14	30	274	40.7	10
	29	15	0	314	36.9	15
	16	15	0	343	35.0	15
	14	13	0	478	36.4	13

[a]Most embryos in this treatment group had pierced the shell membrane.

APPENDIX I continued

Treatment Group	Group Number	C_T ppm	Percent Hatch Control= 100%	N	Exposure Day 12			Exposure Day 19		
					C_2 ppm	Temp °C	Observed Mortality	C_3 ppm	Temp °C	Observed Mortality
Chickens 2 exposures	4	13	106[b]	14	8	32.6	0	5	33.4	0
	42	26	98	14	15	36.9	0	11	38.6	0
	25	45	106	14	13	39.4	0	32	35.0	0
	26	58	98	14	28	36.1	0	30	37.3	1
	46	67	92	15	44	34.4	0	23	37.4	1
	6	85	91	14	39	31.8	0	46	35.9	0
	49	136	85	15	85	37.7	0	51	39.0	2
	31	186	98[c]	14	77	38.6	0	109[c]	37.9	1
	34	186	83	14	77	38.6	1	109	37.9	2
	9	186	8	14	51	39.6	1	137	38.0	12
	17	240	82	13	121	35.6	0	119	36.6	3
	21	273	68	14	132	35.4	0	141	34.9	5
	36	349	0	13	219	37.5	0	130	38.9	13
	39	423	7	15	219	37.5	2	204	37.8	12
	12	752	0	15	428	37.6	13	274	40.4	2

[b] 3 of 14 chicks pipped the wrong end of the shell.
[c] most chicks in this treatment group had pipped the shell before the final exposure.

APPENDIX I continued

Treatment Group	Group Number	C_T ppm	Percent Hatch Control = 100%	N	Exposure Day 4			Exposure Day 12			Exposure Day 19		
					C_1 ppm	Temp °C	Observed Mortality	C_2 ppm	Temp °C	Observed Mortality	C_3 ppm	Temp °C	Observed Mortality
Chickens 3 exposures	24	27	97	12	14	37.3	0	5	37.7	0	8	38.4	1
	43	33	97	13	6	37.9	0	16	36.9	0	11	38.6	1
	28	46	97	13	13	36.9	0	13	39.5	1	30	37.3	0
	47	92	106	14	25	37.0	0	44	34.4	0	23	37.4	0
	15	112	106	13	46	35.9	0	49	37.7	0	17	38.2	0
	7	116	81	13	34	38.6	0	40	38.0	0	40	35.9	3
	50	205	48	14	69	38.7	0	85	37.7	0	51	39.0	7
	18	321	48	13	78	38.3	0	124	35.6	2	119	36.6	6
	10	330	17	12	121	34.0	NA[d]	101	42.8	NA	108	38.1	NA
	32	375	83	14	189	36.0	0	777	38.6	1	109	37.9	2
	35	375	22	14	189	36.0	2	77	38.6	2	109	37.9	8
	22	410	14	15	137	39.3	2	132	35.4	7	141	34.9	4
	37	505	0	14	156	39.4	2	219	37.5	11	130	38.9	1
	40	505	0	11	156	39.4	2	219	37.5	6	130	38.9	3

[d]not available; data not able to be collected owing to technical difficulty.

APPENDIX I continued

Treatment Group	Group Number	C_T ppm	Percent Hatch Control= 100%	N	Exposure Day 20		
					C_1 ppm	Temp °C	Observed Mortality
Quail 1 exposure	7	4	102	11	4	36.6	1
	1	5	104	13	5	36.5	0
	13	6	112	11	6	38.3	0
	19	27	103	12	27	36.4	1[d]
	46	30	95	13	30	33.2	1
	37	30	96	14	30	35.0	2
	25	34	82	15	34	36.7	4[d]
	42	51	104	14	51	36.3	1
	22	91	102	11	91	35.7	0
	34	104	103	12	104	35.9	1[d]
	16	114	86	13	114	35.8	3
	28	136	72	14	136	36.0	5
	40	182	0	16	182	39.8	16
	10	236	40	14	236	34.9	8
	40	478	0	16	478	37.6	16

[e]one chick upside down in shell.

APPENDIX I *continued*

Treatment Group	Group Number	C_T ppm	Percent Hatch Control= 100%	N	Exposure Day 13			Exposure Day 20		
					C_1 ppm	Temp °C	Observed Mortality	C_2 ppm	Temp °C	Observed Mortality
Quail 2 exposures	8	7	90	14	3	34.8	1	4	36.6	2
	2	13	106	15	8	36.9	0	5	36.5	1
	14	17	98	14	11	35.6	0	6	38.3	2
	32	28	97	13	12	35.8	0	14	38.1	2[f]
	20	55	106	15	28	36.5	0	27	36.4	1
	26	57	88	13	23	36.5	0	34	36.7	3
	43	94	114	11	43	35.8	0	51	36.3	0
	47	97	86	12	67	36.2	1	30	33.2	2
	38	111	91	10	81	37.4	0	30	35.0	1
	35	179	44	13	75	34.5	1	104	35.9	7
	23	262	8	14	171	41.6	1	91	35.7	12
	29	300	30	15	164	37.2	0	136	36.0	11
	17	328	8	14	214	37.9	1	114	36.3	12
	5	342	8	14	160	36.4	8	182	39.8	5
	11	461	0	13	225	38.2	8	236	34.9	5

[f] chick malposition in egg.

APPENDIX I continued

Treatment Group	Group Number	C_T ppm	Percent Hatch Control = 100%	N	Exposure Day 5			Exposure Day 13			Exposure Day 20		
					C_1 ppm	Temp °C	Observed Mortality	C_2 ppm	Temp °C	Observed Mortality	C_3 ppm	Temp °C	Observed Mortality
Quail 3 exposures	9	11	115	10	4	34.9	0	3	34.8	0	4	36.6	0
	3	19	115	10	6	36.6	0	8	36.9	0	5	36.5	0
	15	20	81	17	3	35.8	0	11	35.6	0	6	38.3	4
	33	70	106	13	42	39.1	1	12	35.8	0	14	38.1	0
	21	96	106	13	41	36.3	0	28	36.5	1	27	36.5	0
	27	100	71	13	43	38.1	2	23	36.5	0	34	36.7	3
	48	158	74	14	61	37.9	1	67	36.2	1	30	33.2	4
	44	167	90	14	73	37.2	0	43	35.9	0	51	36.3	3
	39	175	46	15	64	36.9	1	81	37.6	0	30	35.1	8
	36	355	0	14	176	37.9	1	75	34.5	5	104	35.9	8
	24	461	9	13	199	37.6	3	171	41.6	10	91	35.7	-
	30	471	0	15	171	38.7	1	164	37.2	8	136	36.0	6
	18	584	0	13	256	38.8	11	214	37.9	2	114	35.9	-
	6	586	0	17	244	38.7	8	160	35.8	9	182	39.8	-
	12	725	0	17	264	36.3	2	225	38.2	15	236	34.9	-

APPENDIX II

Regression equations calculated from chicken and quail mortality studies; y is the percent hatch and x is the SRM exhaust concentration.

Treatment	N	Regression Equation	R^2
Chicken 1 exposure	16	$y = 93.80 + 0.1753x - 0.0027x^2$	0.96
Chicken 2 exposures	15	$y = 94.70 + 0.4299x - 0.0087x^2 + 0.00002x^3$	0.71
Chicken 3 exposures	13	$y = 107.05 - 0.4187x - 0.0082x^2 + 0.00004x^3$	0.90
Quail 1 exposure	15	$y = 101.52 + 0.1130x - 0.0033x^2 + 0.00001x^3$	0.80
Quail 2 exposures	15	$y = 104.39 - 0.3168x - 0.0054x^2 + 0.00002x^3$	0.84
Quail 3 exposures	15	$y = 114.54 - 1.3205x + 0.0029x^2$	0.87

APPENDIX III

Individual egg data compiled from blood gas analyses.

Treatment	Egg Number	Incubation Egg Wt in g	Max Length x Width in mm	Age when bled hrs	Embryo Wt g	pO_2 in mm Hg	pCO_2 in mm Hg	pH
1 exposure 22 ppm 37.3°C	6504[a]	56.232	56.97x42.08	289.2	5.620	109.0	14.5	7.551
	6505	57.657	58.34x42.32	289.5	5.480	94.5	17.6	7.641
	6506	58.157	51.53x41.47	290.0	5.620	95.6	19.1	7.552
	6507	54.949	57.48x41.54	-	5.162	84.3	18.8	7.668
	6508	59.317	58.42x42.58	-	5.384	88.0	18.5	7.642
1 exposure 44 ppm 36.9°C	6101[b]	56.599	56.16x42.69	-	4.503	-	-	-
	6102	60.382	57.58x43.15	282.3	4.425	93.4	19.4	7.651
	6103	53.538	60.15x40.45	282.8	4.312	92.9	19.7	7.603
	6104	52.837	56.88x41.26	283.1	4.887	84.5	18.8	7.621
	6105	55.639	58.45x41.24	283.9	4.855	93.3	17.1	7.650
	6101	55.335	56.37x41.85	284.4	4.197	91.3	19.4	7.661

[a] air bubbles in sample, values not accepted for computation.
[b] air bubbles in sample, values not recorded.

APPENDIX III continued

Treatment	Egg #	Initial Egg Wt in g	Max length x Width in mm	Age when bled hrs	Embryo Wt g	pO_2 in mm Hg	pCO_2 in mm Hg	pH
1 exposure 152 ppm 34.3°C	6201	60.478	60.15x42.80	286.2	5.070	84.4	20.9	7.628
	6202	54.212	55.11x42.40	285.2	5.011	89.0	23.3	7.528
	6203	51.307	55.74x40.69	285.6	5.384	90.1	24.8	7.556
	6204[c]	54.398	56.70x41.79	286.5	4.335	80.9	16.8	7.635
	6205	58.928	56.30x43.26	291.8	4.674	89.8	23.0	7.539
	6206	54.061	57.17x40.98	292.3	4.857	101.2	22.1	7.594
2 exposures 89 ppm (37.1) 105 ppm(39.6)	5902	50.891	54.74x40.78	289.9	5.443	93.2	19.3	7.602
	5903	63.360	60.94x43.73	290.4	5.782	89.8	18.8	7.616
	5904	60.810	59.69x43.03	291.0	4.566	94.0	17.3	7.705
	5905	59.252	58.08x42.34	288.5	5.426	83.8	19.5	7.709
	5906	60.730	58.92x42.88	288.9	5.438	89.9	18.7	7.639

[c]needle plugged, blood transferred to instrument capillary tube under increased pressure.

APPENDIX III continued

Treatment	Egg #	Initial Egg Wt in g	Max length x Width in mm	Age when bled hrs	Embryo Wt g	pO_2 in mm Hg	pCO_2 in mm Hg	pH
2 exposures 68 ppm (38.6°C)	6704	61.940	59.82x42.96	289.6	5.054	78.5	18.5	7.627
	6705	57.186	59.84x41.44	290.1	4.878	88.3	19.4	7.573
36 ppm (38.2°C)	6706	57.631	56.48x42.95	289.9	4.583	87.0	20.3	7.601
	6707	62.488	62.77x42.40	290.4	5.098	89.7	18.2	7.39
	6708	57.639	59.29x42.10	290.7	5.104	91.0	18.7	7.640
3 exposures 24 ppm (33.7°C)	6804	57.626	56.95x42.70	292	4.738	91.6	18.2	7.683
	6805	63.951	62.05x43.17	292	5.387	90.2	19.5	7.670
36 ppm (36.0°C)	6806[d]	58.920	57.95x42.45	293	4.676	95.7	19.9	7.721
29 ppm (36.7°C)	6807	56.941	60.10x41.17	293	5.382	90.7	20.8	7.647
	6808[e]	54.749	56.47x41.23	293	4.985	89.9	22.6	7.584

[d] air bubble in cap tube.

[e] egg temp lowered.

APPENDIX III continued

Treatment	Egg #	Initial Egg Wt in g	Max length x Width in mm	Age when bled hrs	Embryo Wt g	pO_2 in mm Hg	pCO_2 in mm Hg	pH
6 exposures 16 ppm (37.0°C)	6403	61.550	63.58x41.58	287.9	4.068	94.5	24.8	7.551
39 ppm (38.1°C)	6404	61.459	60.32x42.37	285.7	5.655	88.5	19.7	7.212
24 ppm (34.3°C)	6405	59.875	60.19x42.08	283.9	5.529	89.9	25.5	7.577
34 ppm (35.4°C)	6406	54.104	57.20x41.93	284.7	5.535	92.3	19.2	7.384
40 ppm (38.3°C)	6407[f]	63.565	60.76x43.49	287.0	5.403	89.2	19.4	-
36 ppm (38.4°C)	6408	58.859	60.57x41.85	288.5	5.090	91.0	18.8	7.613

[f] sample could not be aspirated into pH chamber.

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